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Weed Management in a Changing World

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BIOLOGICAL PROPERTIES OF A NOVEL PADDY HERBICIDE PYRIMISULFAN

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ABSTRACT

"Pyrimisulfan" has been developed as new herbicide for paddy fields. Pyrimisulfan shows good efficacy against a wide range of weeds - *Echinochloa* spp., sedges, broadleaf weeds and SU-resistant weeds. This compound also shows good efficacy against perennial weeds, such *Bolboschoenus maritimus*, *Eleocharis kuroguwai* and *Sagittaria trifolia*. However, the efficacy is unstable under flooded conditions, because of a relatively low soil adsorption and high water solubility compare with other rice herbicides. We needed to develop a controlled-release formulation for use as a one-shot herbicide in Japan. "Best PartnerTM" has been developed using original formulation technology, to stabilize the herbicidal activity of 'pyrimisulfan' under variable environmental conditions. This product is the first one-shot herbicide for rice containing only one active ingredient.

Keywords: pyrimisulfan, perennial weed, sulfonylurea-resistance, paddy field, one-shot herbicide

INTRODUCTION

The total acreage of paddy rice field in Japan in 2010 was 1.63 million ha (MAFF 2010), the one-shot herbicides for paddy rice field were shipped amount equivalent to the acreage of paddy rice field. The one-shot herbicide was developed in 1980s, initially as a combination of mainly grass herbicides and Sulfonylurea (SU) herbicide. However, biotypes of several weed species that are resistant to SU herbicides have emerged since 1990s (Uchino *et al.* 2007). Currently, SU-resistant weeds are controlled by mixing SU herbicides with other types of chemical compounds (Hamamura *et al.* 2007). Therefore, most of one-shot herbicides currently used contain three or four active ingredients. However, to obtain the category "Specially cultivated rice", by the guidelines of MAFF, the number of pesticides and amount of fertilizer must be halved. In order to decrease the total number of herbicides used, the herbicides were required to have a wide usage spectrum.

Pyrimisulfan(2'-[(4,6-dimethoxypyrimidine-2-yl)(hydroxyl)methyl]-1,1-difluoro-6'-(methoxymethyl)methanesulfonanilide), developed by Kumiai Chemical Industry Co., Ltd. and K-I Chemical Research Institute Co., Ltd., is a sulfonanilide herbicide for paddy fields (Yoshimura *et al.* 2011). It is effective against a wide range of weeds including grass weeds, and has high selectivity between weeds and transplanted rice. In this study, we determined the biological properties of pyrimisulfan.

MATERIALS AND METHODS

Chemicals and formulations

Pyrimisulfan (Figure 1) was prepared as a 10% wettable powder (WP) and a 0.67% granule (GR). 0.67% GR is the controlled-release formulation for one-shot herbicide, sold as Best Partner TM 1kg granule in Japan.

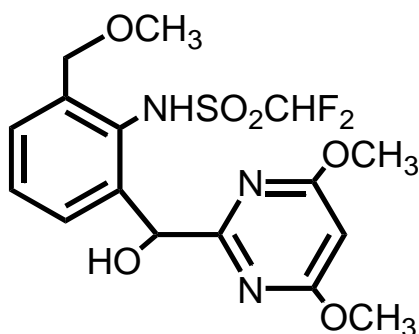


Figure 1. Chemical structure of pyrimisulfan

Weeds

The following annual weeds; *Echinochloa oryzicola* (ECHOR), *Monochoria vaginalis* var. *plantaginea* (MOOVP), *Schoenoplectus juncooides* subsp. *Ohwianus* (SCPJO), and several broadleaf weeds, and the perennials; *Cyperus serotinus* (CYPSE), *Bolboschoenus maritimus* (SCPPL), *Eleocharis kuroguwai* (ELOKU), *Sagittaria pygmaea* (SAGPY) and *S. trifolia* (SAGTR) were used. Sulfonylurea resistant biotypes of MOOVP and SCPJO were collected from paddy fields where SU herbicides were repeatedly used.

Dose response of pyrimisulfan in ECHOR, SU-resistant MOOVP and SU-resistant SCPJO

Trials were conducted in the laboratory. 60 ml of clay loam soil sterilized by autoclaving (121°C, 20 minutes) was placed in 450 ml plastic cups and 200ml of water added. Pre-diluted 10% WP of pyrimisulfan was then added at 0, 0.2, 1.0, 2.0, 4.0, 8.0, 12, 16, 20, 40, 80 and 120 ppb. After application, the weeds were grown in a growth chamber at 30°C in a 16-hour-light and 8-hour-dark cycle. The trial was conducted with four replications, and herbicidal activity was evaluated visually using a scale of 0 (no effect) to 100 (complete kill) at 15 days after treatment.

The half maximal inhibitor concentration (IC₅₀) was calculated by non-linear regression analysis using KyPlot™ 5.0 software (KyensLab Inc.). The data were fitted to the log-logistic model: $y = (a-d)/(1+(x/c)^b)+d$ (a=upper limit, b=slope, c=dose giving 50 % response, d=lower limit) (Seefeldt *et al.* 1995). IC₉₀ was determined using this equation.

Efficacy of 10% WP and 0.67% GR (release control formulation) of pyrimisulfan on paddy weeds as pre-emergence application

This trial was conducted in a greenhouse at Shizuoka Prefecture, Japan. Weeds were sown in a plastic pot (200 cm²) containing clay loam soil. Water was added to a depth of 4 cm above the soil surface. Pre-diluted 10 % WP or 0.67 % GR of pyrimisulfan was applied to the flooded surface at one day after sowing. The following two water management conditions were set up: flooded condition and overflow condition. After 40 or 41 days of application, weed control level was evaluated visually using a scale of 0 (no effect) to 100 (complete kill).

Efficacy of 0.67% GR (release control formulation) of pyrimisulfan on paddy weeds at early post-emergence application

This trial was conducted in the same greenhouse as trial number 4. Weeds were sown in a plastic pot (100 cm² or 200 cm²) containing clay loam soil. Water was added to a depth of 4 cm above the soil surface, and 0.67 % GR of pyrimisulfan was applied to the flooded surface early post-emergence. The same two water management conditions were set up: flooded condition and overflow condition. Between 31 and 45 days after application, weed control level was evaluated visually using a scale of 0 (no effect) to 100 (complete kill).

RESULTS

IC₅₀ and IC₉₀

IC₅₀ of pyrimisulfan was 4.8 ppb for ECHOR, 6.7 ppb for SU resistant MOOVP and 2.9 ppb for SU resistant SCPJO, and IC₉₀ was 15.6 ppb, 15.7 ppb and 9.6 ppb respectively (Figure

2). The sensitivity to pyrimisulfan was the highest in SU resistant SCPJO. There was a difference in dose responses between ECHOR and SU resistant MOOVP at IC_{50} : ECHOR was more sensitive but at IC_{90} , there was no difference in sensitivity.

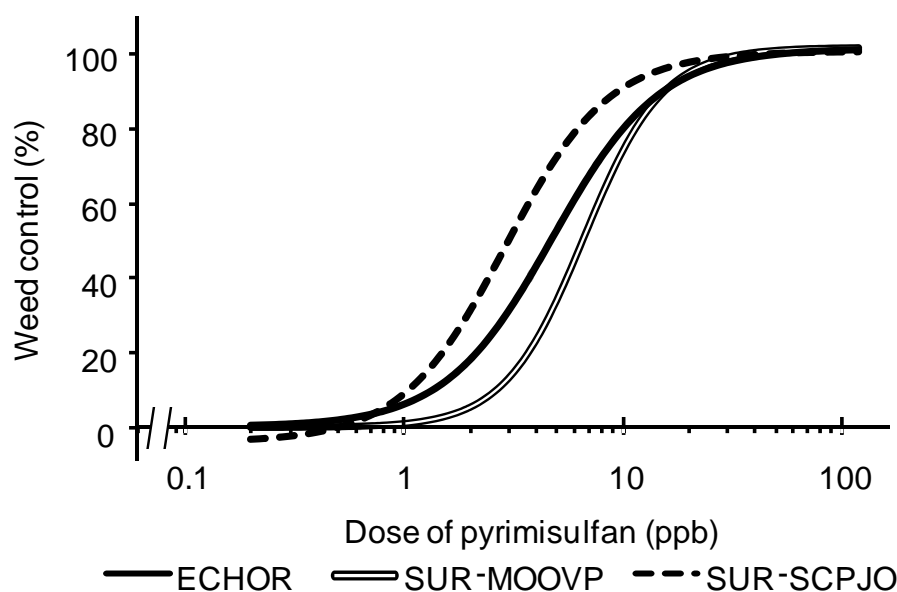


Figure 2. Pyrimisulfan dose response for visual rating data of ECHOR, SUR-MOOVP and SUR-SCPJO. Rating data were 15 days after application.

Efficacy of 10 % WP of pyrimisulfan on paddy weeds at pre-emergence application

Pyrimisulfan 50 g a.i./ha showed high herbicidal activities on 14 weeds including ECHOR, annual broadleaf, perennial sedge, perennial broadleaf and SU-resistant weeds in the flooded condition (Figure 3). However, in the overflow conditions, pyrimisulfan did not show sufficient efficacy at 50 g a.i./ha on ECHOR, perennial weeds and SU-resistant weeds.

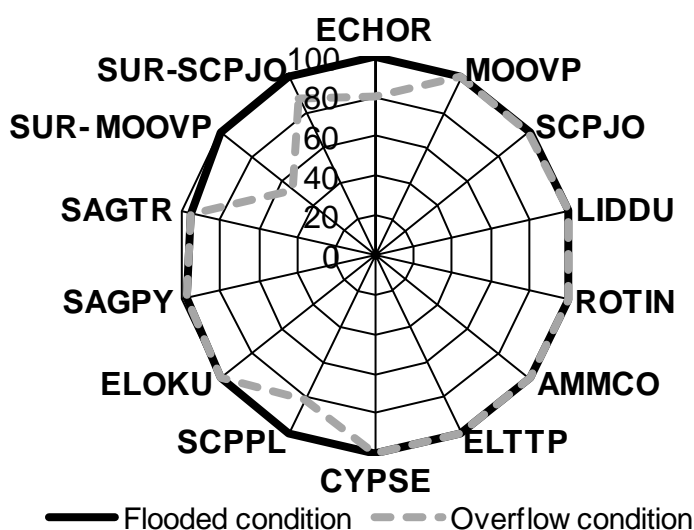


Figure 3. Efficacy of 10 % WP of pyrimisulfan at pre-emergence application. Weed control level was evaluated after 40 or 41 days of application.

Efficacy of 0.67 % GR of pyrimisulfan on paddy weeds at pre-emergence application

Pyrimisulfan 67g a.i./ha showed high herbicidal activities on 14 weeds in the flooded condition (Figure 4). Similarly in the overflow condition, it showed high activity.

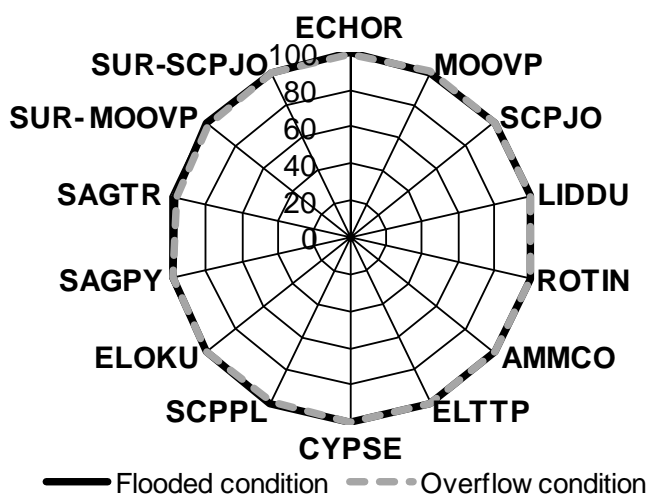


Figure 4. Efficacy of 0.67% granule of pyrimisulfan (controlled-release formulation) at pre-emergence application. Weed control level was evaluated after 40 or 41 days of application.

Efficacy of 0.67 % GR of pyrimisulfan on paddy weeds at early post-emergence application

Pyrimisulfan 67 g a.i./ha showed high herbicidal activities (90 % or over) on 10 weeds in the flooded and overflow conditions (Figure 5).

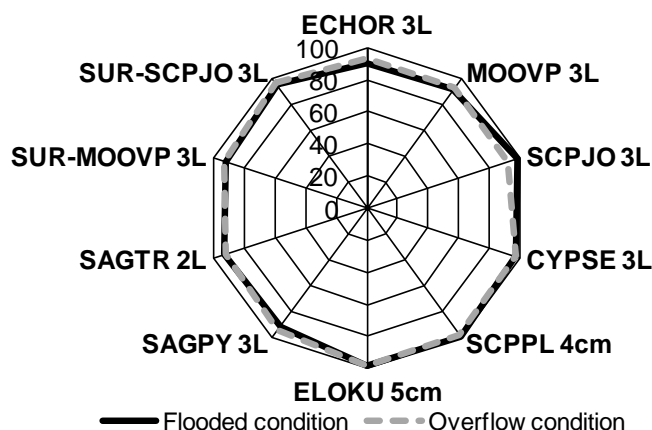


Figure 5. Efficacy of 0.67 % granule of pyrimisulfan (controlled-release formulation) at early post-emergence application. Weed control level was evaluated between 31 and 45 days after application.

DISCUSSION

Pyrimisulfan was discovered by redesigning PC-herbicides through replacing the oxy-bridge with carbonyl-bridge and carboxyl group with sulfonamide group. It was presumed to be an acetolactate synthase (ALS) inhibiting herbicide (Yoshimura *et al.* 2011). SU herbicides, which are ALS inhibitors that have already been used in paddy fields, are not sufficiently effective against *Echinochloa* spp. and SU-resistant weeds, whereas pyrimisulfan at 50 g a.i./ha showed high efficacy against these weeds. In addition, pyrimisulfan exhibited excellent herbicidal effects on other troublesome perennial weeds, such *Bolboschoenus maritimus*, *Eleocharis kuroguwai* and *Sagittaria trifolia*. Therefore, this indicated that pyrimisulfan has potential to control weeds with fewer compounds than conventional herbicides.

On the other hand, the efficacy of pyrimisulfan declined under the overflow condition, because of a relatively low soil adsorption and high water solubility compare with other rice herbicides (Figure 3). We needed to develop a controlled-release formulation for a one-shot herbicide in Japan. "Best PartnerTM" 1kg granule (containing 0.67% pyrimisulfan) was developed with our original formulation technology to stabilize the herbicidal activity of 'pyrimisulfan' under variable environmental conditions. Its activity is less likely to be affected by overflow and it showed high herbicidal efficacy (Figure 4 and 5).

Pyrimisulfan was considered to have sufficient performance to control the troublesome paddy weeds with one active ingredient, and the controlled-released product is the first one-shot herbicide for rice containing only one active ingredient.

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SCREENING PARTHENIUM WEED (*Parthenium hysterophorus* L.) SEEDLINGS FOR THEIR ALLELOPATHIC POTENTIAL

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ABSTRACT

A laboratory bioassay called 'the relay seeding technique' was adapted to detect the presence of growth-altering allelopathic chemicals coming from parthenium weed (*Parthenium hysterophorus* L.) seedlings and to determine their effects upon the growth of a number of grass and broadleaf species. The results showed a species-specific response, with either increased or decreased root or shoot length when these other species were grown with parthenium weed seedlings. In the case of curly windmill grass (*Enteropogon acicularis* L.) and buffel grass (*Cenchrus ciliaris* L.) there was a tendency to reduce the root length when they were grown with parthenium weed. On the other hand, Rhodes grass (*Chloris gayana* L.) seedlings promoted root elongation when grown with parthenium weed. Preliminary results suggest that parthenium weed seedlings might be capable of interfering with the growth of certain species even at the very early stages of growth and this would contribute to its invasive capacity.

Keywords: parthenium weed, allelopathy, bioassay, seedling growth

INTRODUCTION

The allelopathic properties of parthenium weed (*Parthenium hysterophorus* L.) and its ability to inhibit the growth and development of other species is well documented (Kanchan and Jayachandra 1980; Picman *et al.* 1980; Picman and Picman 1984; Mersie and Singh 1988; Fuente *et al.* 2000; Tefera 2002; Sinha and Singh 2004; Singh *et al.* 2005; Belz *et al.* 2007). This is largely attributed to the presence of parthenin - a sesquiterpene lactone of pseudoguanolide nature found in various parts of the weed (Batish *et al.* 2002). The parthenin can leach out from these plant parts when alive or dead. Parthenium weed also releases phenolic acids from its roots and leaves (Valliappan and Towers 1988) as well as from its achenes (Picman and Picman 1984) and from decaying plant residues in the soil (Kanchan and Jayachandra 1980; Mersie and Singh 1988). These water soluble compounds have also been identified as being involved in the allelopathic response of this weed.

The aim of this study was to adapt a laboratory bioassay called 'the relay seeding technique' (Navarez and Olofsdotter 1996) to determine the presence of growth-

suppressing allelopathic metabolites coming from the roots of parthenium weed seedlings and to demonstrate their effects upon the growth of the seedlings of a number of grass and broadleaf species.

MATERIALS AND METHODS

The laboratory bioassay was undertaken in six steps. Firstly, 20 washed parthenium weed seeds were sown in each of six Petri dishes and 5 mL of distilled water added. The bottom of the Petri dishes were previously covered by an autoclaved absorbent material (Geowool) and two 9 cm diameter Whatman No.1 filter papers placed on top. Secondly, 5 days after sowing, parthenium weed seedlings were thinned down to leave 10 relatively uniform seedlings per Petri dish. Thirdly, 8 days after sowing, 20 washed seeds of a second species (i.e. either buffel grass - *Cenchrus ciliaris* L., Rhodes grass - *Chloris gayana* L., desert bluegrass - *Bothriochloa ewartiana* L., curly windmill grass - *Enteropogon acicularis* L., cotton panic grass - *Digitaria brownii* Hughes, weeping grass - *Microlaena stipoides* R.Br., dwarf amaranth - *Amaranthus macrocarpus* Benth., mikania vine - *Mikania micrantha* Kunth and cinderella weed - *Synedrella nodiflora* L.) were sown next to the 10 parthenium weed seedlings. On the same day, 40 filled washed seeds of the second species were sown alone in six Petri dishes as control treatments.

Immediately after the sowing of the second species, the seeds of both species were covered with 2.5 g of white fine granular Perlite to help support the weight of the growing seedlings. Five days after the sowing of the second species, its seedlings were thinned down to leave 10 relatively uniform seedlings per Petri dish. To maintain an adequate water supply to the seedlings for the duration of the study, each dish was connected to a small container filled with distilled water via a filter-strip. Additional distilled water was added to each dish when levels began to fall. These Petri dish units were distributed randomly inside an incubator under a day/night temperature regime of 25/20°C with a 12 hour of photoperiod. Lastly, 27 days after the initial sowing of parthenium weed, all seedlings were gently removed from the Petri dishes and their root and shoot lengths measured using a ruler and biomass collected following tissue drying in an oven at 60 ± 1 °C for ca. 72 hours. The whole experiment was repeated for three of the species (i.e. buffel grass, Rhodes grass and curly windmill grass) to validate previous results.

The statistical analysis was performed by an Analysis of Variance (ANOVA) using the General Linear Model procedure in Minitab, version 16 (Minitab Inc., USA) for one trait at a time (three separate ANOVAs). The data for root and shoot length was quite variable within Petri dishes so averages for each repetition for each trait were used for the analysis.

RESULTS

The results showed a species-specific response, with either increased or decreased root and shoot lengths being observed when seedlings were grown with parthenium weed seedlings. When considering the average shoot length and dry matter changes observed there was no significant differences detected. However, there was a trend towards a longer shoot length for Rhodes grass, buffel grass, weeping grass (only species with a significant response, $P < 0.05$) and dwarf amaranth when growing with parthenium weed.

Conversely, the shoot growth of desert bluegrass, cotton panic grass, curly windmill grass and mikania vine was inhibited when growing with parthenium weed.

The ANOVA for the average root length showed a significant effect associated with the treatment. Even though the differences between growing with or without parthenium weed seedlings for each species were not statistically significant, results from the two screenings showed a consistent tendency for the recurrent species across bioassays (i.e. buffel grass, Rhodes grass and curly windmill grass). In the cases of curly windmill grass, buffel grass and dwarf amaranth there was a tendency towards reduced root length when growing with parthenium weed (Figure 1). On the other hand, the root length of Rhodes grass, weeping grass (only species with a significant response, $P < 0.05$) and cinderella weed was longer when growing with parthenium weed (Figure 1).

With regard to dry matter production, this trait was reduced on average across all species when they were grown with parthenium weed in the first screening. However, the interaction of species \times treatment was not significant, thus no particular species has been found that is significantly affected. The interaction was not significant for the second screening either and the trends for the repeated species were not consistent across screenings.

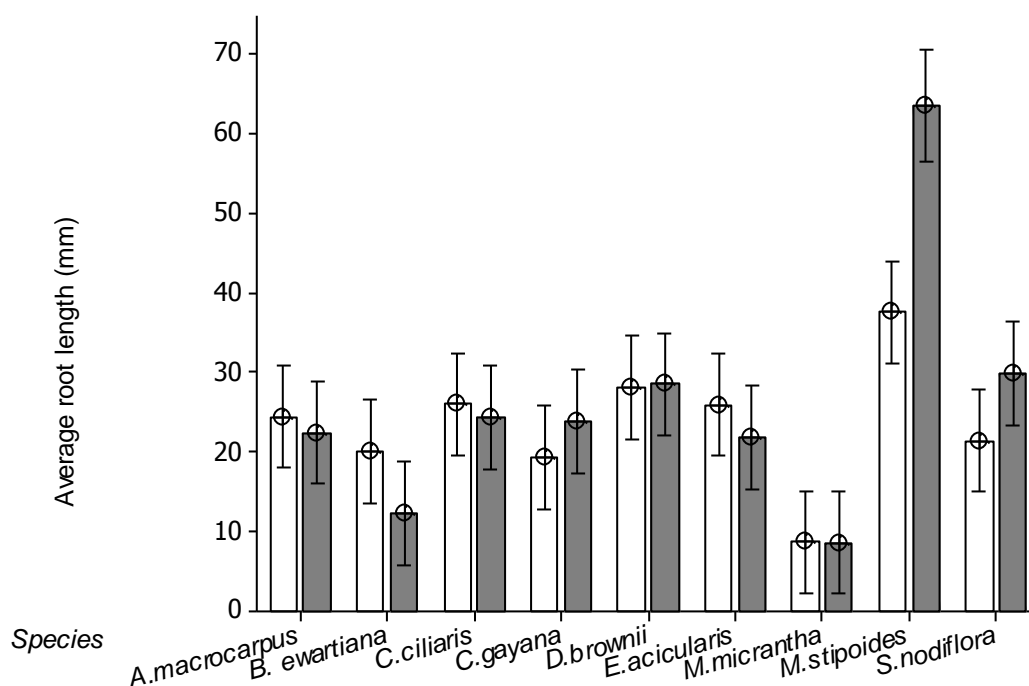


Figure 1. Average root length (mm) for the nine tested species growing alone (\square) or with parthenium weed (\blacksquare). Error bars represent 95% confident interval calculated using pool standard error.

DISCUSSION

As the employed technique (Navarez and Olofsdotter 1996) ensures no competition occurs between species for the available resources (i.e. light, water and space), the growth suppression/stimulation seen in this test is believed to be due to an allelopathic interaction. The preliminary results suggest that parthenium weed might be imposing an allelopathic stress on the growth of certain test species and this can result in either an increase or a decrease in their growth rate. Differential species sensitivity to parthenium weed allelochemicals have been previously reported (Mersie and Singh 1987; Batish *et al.* 2002). Most likely, water soluble compounds such as phenolic acids, previously reported as being involved in the allelopathic response of this weed (Valliappan and Towers 1988), are being released by parthenium weed's roots and causing these growth responses.

Parthenium weed seedlings might be interfering with the growth of certain species even from the very early stages of their development, which would contribute to the invasive capacity of this species. However, the released amounts of these secondary metabolites at such early growth stages of the weed did not reach concentrations high enough to detect any statistically significant effects in these experiments.

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INTEGRATED WEED MANAGEMENT IN GROUNDNUT (*Arachis hypogea*)

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ABSTRACT

An experiment on integrated weed management in groundnut (*Arachis hypogea*) was conducted at Department of Agronomy during 2009-10. Ten treatments which included three herbicides viz., Pendimethalin, quizolofop ethyl, Imazethapyr along with mechanical weeding were studied in randomized block design with three replications. Two hand weeding at 15 and 30 days after sowing were effective to reduce weed count and weed biomass, and increased WCE and thus increased developed pods and pod yield. Complete weed free condition recorded highest dry pod yield (1786 kg ha⁻¹). Pre emergence application of Pendimethalin 1.0 kg a.i./ha followed by one hand weeding at 15 days after sowing resulted in 10.8 pods/ plant as against 4.97 pods/ plant in unweeded control. In these treatments nodules per plant were 60.0 vs 37.5 in unweeded plots, with pod yield 1658 kg ha⁻¹ vs 677 kg ha⁻¹ in unweeded control. Pre-emergence spray of Pendimethalin @ 1.0 kg a.i./ha followed by post emergence Imazethapyr @ 75 g a.i./ha at 15 days after sowing increased pod yield (1255 kg ha⁻¹) as compared to unweeded control. This treatment increased shelling % and kernal weight. Pendimethalin @ 1.0 kg a.i./ha+1HW at 15 days after sowing increased nutrient uptake (85 kg N + 9 kg P₂O) as compared to weedy check (45 kg N + 2.8 kg P₂O). Application of Pendimethalin resulted in 16.0X10⁴ cfu g⁻¹ soil fungal count, 15.67X10⁶ cfu g⁻¹ soil actinomycetes count and 20.33X10⁴ cfu g⁻¹ soil bacterial count per gram of soil, an increase compared to unweeded control. Cultural weeding resulted in higher microbial counts compared to herbicide application.

Key words Pre-emergence Pendimethalin, fungal count, Actinomycetes, bacterial count, pod yield, hand weeding.

INTRODUCTION

Groundnut (*Arachis hypogea*) is a valuable oilseed, and accounts for 33% area and 45% production in India. India ranks first among groundnut growing countries in the world with 6.74 M.ha. area and 7.99 MT production. Integrated weed management in groundnut has great importance as groundnut suffers heavily due to weed competition in the early stage because of its short structure and initial slow growth. Up to 70% reduction in groundnut yield is reported by Dev Kumar and Giri (1998). Weeds compete with crop for soil moisture, nutrients and light and reduce the yield. They also harbour and serve as alternative host for pest and diseases. The critical period of crop weed competition in groundnut was observed to be 4 to 8 weeks after sowing (Santelmann and Hill 1969), as groundnut is naturally short with slow seedling emergence and initial growth. The loss in yield of groundnut pods due to weed competition ranged from 30 to 40% (Chandra Singh and Gupta 1973). Nutrient losses due to crop weed competition were 38.8, 9.2 and 23.3 N, P and K kg ha⁻¹ respectively (Naidu *et al.* 1982).

Mechanical and cultivation weeding are economical under Indian condition, but the time of application is important. Field situations such as continuous rains do not permit timely mechanical operations. Herbicide gives timely and effective control of weeds and traditional methods give better aeration and soil condition along with weed control. Therefore use of herbicide alone or in combination with cultivation has become a necessity to control weeds. With this in view, an investigation of integrated weed management in groundnut was conducted to study the relative efficacy of herbicides to control weeds, and to study the nutrient uptake of weeds.

MATERIALS AAND METHODS

The experiments were conducted at Agronomy farm, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.), India during 2009-10. The treatments, comprising three herbicides, cultural method of weed control and integration of these, were replicated three times in randomized block design. The herbicides used were a pre-emergence application of Pendimethalin @ 1.0 kg a.i./ha + one hand weeding at 15 DAS, post emergence Emazethapyr @ 75 g a.i./ha at 15 DAS, and post emergence of Quizolpoph ethyl @ 50 g a.i./ha at 15 DAS, plus three cultural treatments with two controls, carried out in groundnut during rainy season. Details of treatments are given in Table 1. Plot size was 3.6mX4.0m and 2.7mX3.0m gross and net respectively. The crop was sown at 30X10 cm spacing with 100 kg seed rate and fertilizer dose of 25:50:0 NPK kg/ha. The weed control efficiency was calculated as

$$WCE = \frac{wpc - wpt}{wpc} \times 100$$

where,

wpc = weed population in unweeded plot

wpt = weed population in treated plot

The weed index was derived as

$$W1 = \frac{x - y}{x}$$

where,

x = yield from weed free treatment

y = yield from weed treatment for which w1 is to be calculated.

The microbial count, viz., bacterial count, actinomycetes and fungal counts, were recorded from one gram of soil before and after herbicide treatment. The nutrient uptake was calculated from the dry matter produced by the plant.

RESULTS

Weed Studies

The crop was infested with both monocot and dicot weeds. Of monocot weeds, *Cynodon dactylon*, *Ischaemum pilosum* and *Digitaria sanguinalis* were dominant while for the dicots *Parthenium hysterophorus*, *Convolvulus arvensis*, *Achyranthes aspera*, *Phyllanthus niruri*, *Euphorbia hirta*, *Digera arvensis* and *Tridax procumbens* were dominant. Pre-emergence application of Pendimethalin followed by hand weeding was effective to control weeds at the early stage of crop growth, and the dicot weeds were 13.67 per sq.m. as against 19.00 in unweeded control. Monocot weeds recorded were 17.33 as against 25.67 per sq.m.

Pendimethalin @ 1.0 kg a.i./ha as pre-emergence followed by Imazethapyr @ 75 g a.i./ha resulted in a 14.72 weed index and 51.17 % weed control efficiency (Table1).

Table 1. Effect of treatments on weed index and weed control efficiency.

Treatments	Weed Index (%)	Weed Control Efficiency (%)	
		Monocot	Dicot
T ₁ -Unweeded (Control)	62.09	Control	Control
T ₂ -Weed free (Check)	---	100	100
T ₃ -PE Pendimethalin @ 1.0 kg a.i./ha + one hand weeding at 15 DAS	7.17	55.80	52.64
T ₄ -PoE Quizolofop ethyl @ 50 g a.i./ha at 15 DAS	30.96	52.32	44.75
T ₅ - PoE Emazethapyr @ 75 g a.i./ha at 15 DAS	29.73	51.17	42.15
T ₆ - PE Pendimethalin @ 1.0 kg a.i./ha + T ₄	25.36	51.17	52.64
T ₇ - PE Pendimethalin @ 1.0 kg a.i./ha + T ₅	14.72	52.32	42.15
T ₈ - Two weeding at 15 DAS and 30 DAS	2.52	62.78	73.72
T ₉ - Two hoeing at 15 DAS and 30 DAS	9.46	59.29	55.25
T ₁₀ -One weeding at 15 DAS and one hoeing at 30 DAS	7.58	60.48	57.93

Pod yields

Table 2. Pod yield, haulm yield and shelling percentage as influenced by various treatments.

Treatments	Pod yield (kg ha ⁻¹)	Haulm (kg ha ⁻¹)	Shelling percentage
T ₁ -Unweeded (Control)	677	2272	68.66
T ₂ -Weed free (Check)	1786	2314	73.45
T ₃ -PE Pendimethalin @ 1.0 kg a.i./ha + one hand weeding at 15 DAS	1658	2281	71.71
T ₄ -PoE Quizolofop ethyl @ 50 g a.i./ha at 15 DAS	1233	2283	72.04
T ₅ - PoE Emazethapyr @ 75 g a.i./ha at 15 DAS	1255	2252	72.60
T ₆ - PE Pendimethalin @ 1.0 kg a.i./ha + T ₄	1333	2305	70.53
T ₇ - PE Pendimethalin @ 1.0 kg a.i./ha + T ₅	1523	2319	71.34
T ₈ - Two weeding at 15 DAS and 30 DAS	1741	2281	73.65
T ₉ - Two hoeing at 15 DAS and 30 DAS	1617	2301	71.99
T ₁₀ -One weeding at 15 DAS and one hoeing at 30 DAS	1651	2294	72.81
S.E. (m)±	0.51	0.19	0.60
C.D. at 5 %	1.50	NS	1.79

The data presented in Table 2 indicate that pod yield of groundnut was influenced significantly by the various treatments. The absolute weed free condition produced the maximum pod yield (1786 kg ha⁻¹). Hand weeding twice at 15 and 30 DAS resulted in significantly more pod yield (1741 kg ha⁻¹) than the weedy check (677 kg ha⁻¹). Pre-emergence application of Pendimethalin @ 1.0 kg a.i./ha produced pod yield of 16.58 kg ha⁻¹, comparable to the complete weed free condition and on par with cultivation method of weed control. Post emergence application of Quizolofop ethyl @ 50 g a.i./ha at 15 DAS resulted in 1233 kg ha⁻¹ of dry pod yield, comparable to other herbicides and also to cultivation practices. Haulm yield, shelling % and 100 kernal weight were improved in herbicide treatments which were comparable to the cultural method of weed control.

Nutrient uptake

Table 3. Total nutrient uptake as influenced by various treatments.

Treatments	Total nutrient uptake by plant	
	N (kg ha ⁻¹)	P ₂ O (kg ha ⁻¹)
T ₁ -Unweeded (Control)	45.79	2.83
T ₂ -Weed free (Check)	85.37	9.35
T ₃ -PE Pendimethalin @ 1.0 kg a.i./ha + one hand weeding at 15 DAS	77.42	8.41
T ₄ -PoE Quizolofop ethyl @ 50 g a.i./ha at 15 DAS	55.24	4.58
T ₅ - PoE Emazethapyr @ 75 g a.i./ha at 15 DAS	59.02	5.93
T ₆ - PE Pendimethalin @ 1.0 kg a.i./ha + T ₄	61.44	6.49
T ₇ - PE Pendimethalin @ 1.0 kg a.i./ha + T ₅	65.44	7.13
T ₈ - Two weeding at 15 DAS and 30 DAS	84.59	8.77
T ₉ - Two hoeing at 15 DAS and 30 DAS	66.31	6.41
T ₁₀ -One weeding at 15 DAS and one hoeing at 30 DAS	73.09	7.79
S.E. (m)±	0.10	0.04
C.D. at 5 %	0.29	0.11

Maximum uptake of nutrient (NP) was observed with weedfree condition followed by cultural method of weed control. Pre-emergence application of Pendimethalin followed by hand weeding recorded maximum nutrient uptake (77.42 N : 8.41 P₂O kg ha⁻¹) which was comparable to other herbicides used (Table 3).

Microbial studies

The data presented in Table 4 indicated that cultural method of weed control and complete weed free condition recorded more bacterial, actinomycetes and fungal counts. Pendimethalin 1.0 kg a.i./ha followed by handweeding improved the fungal count (16X10⁴ cfu g⁻¹), actinomycetes count (15.67X10⁶ cfu g⁻¹) and bacterial count (20.33X10⁷ cfu g⁻¹) per gram of soil which was comparable to other herbicides. The lowest values for these colonies were observed in the weedy chec

Table 4. Microbial count as influenced by different treatments.

Treatments	Fungal count (X10 ⁴ cfu g ⁻¹)	Actinomycetes count (X10 ⁶ cfu g ⁻¹)	Bacterial count (X10 ⁷ cfu g ⁻¹)
	After spraying	After spraying	After spraying
T ₁ -Unweeded (Control)	8.33	10.00	14.33
T ₂ -Weed free (Check)	21.00	19.33	25.33
T ₃ -PE Pendimethalin @ 1.0 kg a.i./ha + one hand weeding at 15 DAS	16.00	15.67	20.33
T ₄ -PoE Quizolofop ethyl @ 50 g a.i./ha at 15 DAS	13.67	14.67	18.33
T ₅ - PoE Emazethapyr @ 75 g a.i./ha at 15 DAS	13.67	14.67	18.67
T ₆ - PE Pendimethalin @ 1.0 kg a.i./ha + T ₄	14.67	15.33	19.67
T ₇ - PE Pendimethalin @ 1.0 kg a.i./ha + T ₅	15.33	15.67	19.33
T ₈ - Two weeding at 15 DAS and 30 DAS	19.67	17.67	24.33
T ₉ - Two hoeing at 15 DAS and 30 DAS	18.67	16.67	21.33
T ₁₀ -One weeding at 15 DAS and one hoeing at 30 DAS	19.33	16.67	22.33
S.E. (m)±	0.39	0.36	0.34
C.D. at 5 %	1.16	1.08	1.01

DISCUSSION

Groundnut (*Arachis hypogea*), an important legume crop, is most sensitive to weed infestation. Flowering on groundnut is initiated at 21 days after sowing and this stage is most sensitive to moisture and nutrient stress. Weeds compete for these resources and thus reduce growth and yield of groundnut. Cultural method (cultivation) for weed control is most effective as it pulverises the soil and thus provides better aeration for root proliferation, nodulation and pod development. As evidenced in Table 1 the weed counts were reduced by two weedings at 15 and 30 DAS. Similar results were reported by Sukhadia *et al.* (1998), with pod yield increased by the cultural method of weed control. Pre-emergence application of Pendimethalin 1.0 kg a.i./ha reduced monocot and dicot populations in the early stage of crop growth which permitted better growth of crop, pod bearing and thus finally improved pod yield. Similar observations were reported by Rath *et al.* (1986) who also stated that Pandimethalin @ 1.5 kg a.i./ha was as effective as two hand weedings.

Integrated Weed Management

Pendimethalin 1.0 kg a.i./ha followed by one hand weeding at 15 DAS was more effective to control weeds at the early crop growth stage. Hand weeding allows pulverisation of soil, better aeration, root proliferation, better nodulation and more pod formations, ultimately increasing pod yield (1658 kg/ha). Itnal *et al.* (1993) also showed that pre-emergence application of Pendimethalin 1.0 kg a.i./ha followed by one hand weeding was most effective not only to control weeds but also in obtaining higher pod yield of groundnut. Better crop growth due to early and effective weed control allowed absorption of more nutrient from soil and the nutrient uptake was N 77 kg ha⁻¹ and P₂O 8.40 kg ha⁻¹, comparable to the nutrient uptake with weed control through cultural methods as well as other weedicides, used either as pre-emergence or post emergence sprays. The microbial population was increased by herbicide treatment and was 16X10⁴ cfu g⁻¹ for fungus and 15.67X10⁶ cfu g⁻¹ for actinomycetes per gram of soil. This indicates that herbicide application has no adverse effect on the microbial population.

CONCLUSION

Integrated nutrient management through Pendimethalin 1.0 kg a.i./ha followed by one hand weeding 15 DAS was most effective to control weeds and increase yield of groundnut.

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ALLELOPATHY FOR WEED MANAGEMENT IN WHEAT

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ABSTRACT

Growing alarm concerning the toxicity of synthetic herbicides and evolution of new weed biotypes demands innovative tools for their management. Allelopathy, a naturally occurring phenomenon in agricultural ecosystems, has received enormous consideration in recent years as possible alternative for chemical weed management. We have extensively explored potential allelopathic plants including sunflower, sorghum, mulberry, brassica spp. and eucalyptus for weed management in wheat in a series of studies. We have proved that application of allelopathic crop water extracts in combination with reduced doses of herbicides provide as effective weed control as full herbicide dose in wheat. Now we are focusing on the organic weed management options using allelopathy.

Key words: Allelopathy, weed management, wheat

INTRODUCTION

The need for safe food production has developed steadily over the years as a result of consciousness about food quality and environmental concerns regarding agrochemicals used in agriculture. World wide huge crop losses have are due to heavy weed infestations. The losses associated with weeds in Pakistan exceed Rs. 120 billion on national level (Anonymous, 2005). The weed losses in major cereal crops are in the range of Rs. 40, 30 and 4 billion for rice, wheat and maize, respectively (Anonymous, 2005). Farmers normally rely on quick and effective control measures by using synthetic herbicides which produce several detrimental impacts for human health due to their indiscriminate use (Kohli *et al.*, 1998; Xuan *et al.*, 2004).

In the search for promising weed control methods scientists are investigating various aspects of allelopathy as a means of weed suppression. Crops/plants release chemicals i.e “allelochemicals” which could be utilized for managing weeds (Putnam and Defrank, 1979). Brassicas have been discussed as potential alternative to synthetic herbicides for weed control (Grossman, 1993). Different doses of sorghum water extract (sorhaab) applied as single and multiple foliar sprays at different days after sowing (DAS) suppressed the total weed density at 120 DAS up to 48% (Cheema *et al.*, 1997). Grain yield of wheat was increased by 21% at 1:10 w/v ratio of sorgaab applied twice at 30 and

60 DAS with decreases in weed density and dry weights of 33.6 and 19.92% respectively (Cheema *et al.*, 2000). They also reported no difference between single, double and triple applications of sorgaab in terms of weed control and wheat grain yield. Sorghum water extract (100%) sprayed at 30 DAS significantly suppressed the density of weed species such as broad leaf dock 36%, swine cress 23%, lambsquarters 38%, fumitory 61%. A total weed biomass reduction of 53% was obtained with 100% sorghum water extracts with a corresponding yield increase in wheat of 14% over control (Cheema *et al.*, 1997). It was also observed that sunflower water extracts (100%) inhibited these weeds by 24, 61, 31 and 21% respectively, with total weed dry weight reduced by 51% and wheat yield increased by 7% over control. Mulberry extracts inhibited the seedling growth of bermuda grass more than wheat seedling and interestingly, its foliar spray at 100% significantly inhibited the growth of Bermuda grass and promoted wheat growth (Haq *et al.*, 2010).

These data indicate that sorghum, sunflower and mulberry water extracts used separately inhibit weeds up to 20-50%, which is less than the standard weed control i.e 80% or above; but it is quite possible that when various extracts are tank mixed, they may demonstrate a cumulative effect.

MATERIALS AND METHODS

Soil and Site

The present field study, exploring weed control with different crop aqueous extracts in wheat, was conducted at Agronomic Research Farm University of Agriculture, Faisalabad (31.25° N and 73.09° E), Punjab, Pakistan during the winter of 2008-9. The experimental soil belongs to Lyallpur soil series (Aridisol-fine-silty, mixed, hyperthermic Ustalfic, Haplargid in USDA classification and Haplic Yermosols in FAO classification scheme).

Crop Husbandry

The wheat variety Sehar-2006 was sown on a cultivated seed bed in 22cm spaced rows using a single row hand drill on Dec 4, 2008 using 125 kg ha⁻¹ seed. Fertilizers in the form of urea and diammonium phosphate were applied at 100 and 60 kg ha⁻¹ respectively. Half of the nitrogen and full dose of phosphorus were applied at the time of sowing, while the remaining half of nitrogen was applied with the first irrigation. The first irrigation was given 20 days after sowing and subsequent irrigations were adjusted according to the climatic conditions and need of the crop.

Experimental details

For weed control, a mixture of sorghum, sunflower and mulberry water extracts were applied each at 12, 15, 18 L ha⁻¹. The synthetic herbicide used as a control was Affinity 50

WP (carfentrazone-ethyl 0.75%+isoproturon 50% applied at 1000 g a.i. ha⁻¹) and a weedy check was also maintained as a control. The water extracts were prepared following the procedure of Cheema and Khaliq. (2000) and were applied twice at 40 and 55 days after sowing (DAS). The label dose of Affinity 50 WP was applied at 30 DAS. The volume of the spray (340 L ha⁻¹) was determined after calibration following the Ross and Lembi (1985) procedure. The knapsack hand-sprayer was fitted with a flat fan nozzle maintaining a pressure of 207 kpa.

Measurements

Data for individual and total weed density and biomass in a unit area was recorded 60 DAS using a 0.25 m x 0.25 m quadrat randomly placed at two places in each experimental unit. Weeds were oven dried at 70 °C for 72 hours for the dry weight. Wheat crop was harvested and threshed manually in third week of April, 2009 from individual treatment plots; grain yield was weighed in kilograms and expressed as mega gram per hectare (Mg ha⁻¹). Other yield traits were recorded by standard sampling techniques.

Statistical analysis

Data were analyzed using statistical package MSTATC (Freed and Scott, 1986). Analysis of variance (ANOVA) was performed using Fisher's analysis of variance technique while multiple comparisons among treatment means were made using least significant difference (LSD) test at P<0.05 (Steel *et al.*, 1997).

RESULTS

The predominant weed flora in the field was wild oats, little-seed canary grass, swine cress, lambs quarters and a few plants of field bindweed. All of the weed control treatments substantially reduced density of wild oats as compared to weedy check (Table 1). Maximum inhibition of both narrow and broad leaf weeds was obtained with synthetic herbicide. It was followed by allelopathic water extracts (WE) treatment viz. sorghum + sunflower + mulberry each at 21 L ha⁻¹ at 40+55 DAS and sorghum + sunflower + mulberry water extracts each at 18 L ha⁻¹ at 40+55 DAS (Table 1). These WE treatments (WE 15-12 L ha⁻¹) for wild oats density were statistically comparable, while for little seed canary grass only, the treatment sorghum + sunflower + mulberry at 18-21 L ha⁻¹ was followed by that with synthetic herbicide. The weed dry biomass inhibition was similar to that observed for weed densities (Table 1).

Both of the broadleaf weeds lambs quarters and swine cress were effectively reduced by synthetic herbicide, 95% and 96.3% respectively. The density of lambs quarters in the WE treatments was almost similar to the treatment combination of sorghum + sunflower + mulberry at 15-21 L ha⁻¹ as well as in water extract combination of sorghum + sunflower at

15-21 L ha⁻¹. Maximum density inhibition in lambs quarters 94% and swine cress 91% was achieved by sorghum + sunflower + mulberry each at 21 L ha⁻¹. Weed dry biomass production was reduced in the same way as density by WE treatments (Table 2). Maximum dry biomass inhibition 2.66g (i.e 94.8%) was observed from the herbicide as compared to control treatment. It was followed by WE treatment of sorghum + sunflower + mulberry at 21 L ha⁻¹ (6.11g) with 88% reduction over control treatment (51.18g). The WE treatment of sorghum + sunflower at 15-21 L ha⁻¹ and sorghum + sunflower + mulberry at 21 L ha⁻¹ were statistically equal in reducing dry biomass production by 77-87% (Table 2). The total weed density and dry biomass production was also sufficiently reduced by WE treatments to enhance grain yield. Highest grain yield 3.78 t ha⁻¹ with 29% increase over control was obtained from herbicide treatment. Among the WE treatments, maximum increase in grain yield (3.5 t ha⁻¹ with 19.5% increase over control) was obtained from the treatment combination of sorghum + sunflower + mulberry, each at 18 L ha⁻¹ at 40-55 DAS. The WE treatments sorghum + sunflower each at 18 L ha⁻¹ and sorghum + sunflower + mulberry each at 21 L ha⁻¹ at 40+55 DAS were statistically the same and increased grain yield 16% over control. Other water extracts increased yield but none significantly.

DISCUSSION

Several plant species produce a number of secondary metabolites, and some of these compounds play an important protective role in the plant rhizosphere as allelopathic substances (Duke et al. 2000). Aqueous extract treatments significantly reduced the density and dry weight of the weed species present at the experimental site. Density and dry biomass of wild oats, little seed canary grass, swine cress and lambs quarters was significantly reduced by all combinations of sorghum extract with either of the extracts, with sunflower alone or sunflower + mulberry (Table 1). Inhibition in density and dry weight of both grassy and broad leaved weeds substantially increased with increasing WE dose from 12 to 21 L ha⁻¹. Greater weed control at higher WE rates was maybe due to increased concentration of allelopathic compounds present in sorghum such as m-coumaric acid, caffeic acids, gallic acid, protocatechuic acid, syringic acid, vanillic acid, phydroxybenzoic acid, p-coumaric acid, benzoic acid, ferulic acid (Haskins and Gorz, 1985; Netzly & Butler, 1986; Nimbal *et al.*, 1996); in sunflower α -naphthol, scopolin and annuionones (Wilson and Rice, 1968; Macias *et al.*, 1998, 2002; Anjum and Bajwa, 2005) and in mulberry palmatic acid, ascorbic acid, galic acid and vanilic acid (Haq *et al.*, 2010). The weed density and dry matter production was reduced in parallel with increasing WE dose and also by combining three WE i.e sorghum + sunflower + mulberry instead of sorghum + sunflower (Table 1). These findings are supported by Cheema *et al.* (1997) who reported reduction in weed dry weight with sorghum and sunflower extracts as foliar sprays. Greater decrease in the total weed dry mass in WE treatments with increasing dose showed that higher concentration of allelochemicals may be more effective for weed biomass reduction. The dry matter accumulation reflects the growth behavior of a weed and gives the better indication of weed crop competition than the weed density.

Greater weed dry weight reflects more utilization of soil and environmental resources by the weed at the expense of crop growth. This relationship has been proved by an increase in grain yield with decreasing weed dry biomass. Similar results were reported by Cheema and Khaliq (2000), that 1:10 w/v ratio of sorgaab applied twice at 30 and 60 DAS increased wheat grain yield by 21% with decreases in weed density and dry weights of 44 and 49% over control, respectively. The maximum increase in grain yield over control in water extract treatments was by sorghum + sunflower + mulberry each at 18 instead of 21 L ha⁻¹, showing that at certain range of concentration the allelochemicals may have a negative effect on wheat yield. This effect could be seen in this study in which sorghum + sunflower + mulberry each at 12 L ha⁻¹ produced a similar increase in grain yield over control as obtained by sorghum + sunflower each at 21 L ha⁻¹ (Table 2).

CONCLUSION

Sorghum, sunflower, and mulberry are potent allelopathic crops and a combination of water extracts of these crops, each at 18 L ha⁻¹, can be used as a weed management strategy in modern agriculture, resulting in 87% weed control and 19.5% increase in wheat grain yield.

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INCIDENCE OF QUARANTINE INVASIVE WEED *Solanum carolinense* L. IN DIFFERENT ECOSYSTEMS OF TAMIL NADU

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ABSTRACT

A survey of weed flora in different ecosystems (i.e. cropped, non-cropped, waste disposal and derelict areas) during *kharif* and *rabi* seasons (2008 to 2010) was conducted in eight districts of Tamil Nadu. The focus of the weed survey was to detect new invasive weeds and increase the preparedness for exotic weeds invasions.

Solanum carolinense L., a listed, quarantine weed, was observed in 88 places of different survey spots in eight districts; viz., Coimbatore, Salem, Dharmapuri, Krishnagiri, Dindugal, Nilgiris, Erode and Vellore of Tamil nadu. Plants found during the survey were about one meter tall, armed, with small spines.

Solanum carolinense L., stems were erect and possessed stellate hairs, greenish to purple in color. Leaves were alternate, typically lance-ovate, lobed, with spines on midrib and veins. The inflorescence, an axillary raceme with branches, had compact clusters of flowers. The flowers of *Solanum carolinense* were five lobed and corolla was white to lilac or purple in colour. The flower had stellate pubescence externally and was glabrous internally. It contained five stamens filaments, which were yellowish green in colour. Stigma was dark in colour and the superior ovary was glandular, pubescent and whitish in colour. The deeply lobed calyx (five lobes) and tube was purple green in colour.

The immature fruit was green in colour, turning orange and yellow. The fruit, a single, globular berry, contained 120 to 240 seeds. Seeds were flattened, roundest with a peak yellowish to orange brown. The semi hard, woody stem had hairs and thorns all over the plant, making it difficult for farmers to remove the plant manually.

Key words: Invasive weed, *Solanum carolinense*, Incidence, Tamil Nadu

INTRODUCTION

Wheat is a staple food in many of the large States of India. Wheat is grown extensively and produced in large quantities in Central India and Northern States. During times of scarcity and natural calamities, the area cultivated with wheat is largely affected. In order to offset the short fall in production and consequent distribution, wheat is imported from overseas. The Government of India has stringent plant quarantine measures in order to avoid new pests and diseases and the introduction of new weed seeds, which are alien to India. During food scarcity, some of the phytosanitary measures are relaxed, so as to facilitate the import of food grains in large quantities. Such relaxation of import restrictions, had led to the entry of invasive weed species, such as *Parthenium hysterophorus*, *Solanum carolinense*, *Cenchrus trifloides* and *Viola arvensis* in to India.

Consequent upon the policy decision taken by the Government of India, during the year 2006-07, 6.2 million tonnes of the wheat was imported by the Ministry of Food and Consumer Affairs for the Public Distribution System (PDS) from various countries such as Australia, Russia, Canada, Ukraine, Hungary, France, Kazakhstan, Romania, Bulgaria, Netherlands and Argentina. According to the phyto-sanitary regulation of this order, import of wheat should be free from quarantine weeds listed in Schedule VIII of this Order.

Distribution of whole wheat grains for human consumption with unrestricted distribution throughout the country (i.e. Public Distribution System) with multiple rural outlets, may pose serious threat to the wheat growing areas. Since unrestricted distribution could facilitate the spread of invasive weed seeds, the National Invasive Weed Surveillance Project was formulated and focused on four regulated weed species with the following objectives:

1. to effectively survey and monitor for the early detection of regulated weeds;
2. to create public awareness through campaigns on the weeds and their quarantine status, control / containment and eradication;
3. to initiate containment and the eradication of these weeds; and
4. to strengthen national and regional capacities in invasive weed identification and management.

SURVEY METHODS

As a preliminary study, samples of wheat, which was distributed through public distribution system (PDS), were collected. The source of PDS wheat might be from Food Corporation of India (FCI) godowns spread across the State. The Surveillance Inspectors were facilitated and guided in the process of collecting the wheat samples from FCI godowns in Madurai, Vellore and Coimbatore districts.

Weed survey was designed as a specific survey, focusing on selected regulated weeds. The survey was carried out throughout the year, covering all cropping seasons, and was carried out for two consecutive years. Suitable survey route maps were prepared for each district, covering the potential threat areas. In the State of Tamil Nadu, the National Invasive Weed Surveillance operated at Coimbatore centre undertook the task of surveying in 12 districts of Tamil Nadu.

Three out of twelve districts were surveyed by one Surveillance Inspector, who surveyed around 10 locations in the survey route covering approximately 100 km per day. At each location, four plots (one square meter each), which comprised 10 from cropped area, 10 from non-cropped area and 10 from the garbage area were surveyed for the incidence of regulated quarantine weeds. The sampling was done in four plots to cover one square meter area and the density of weeds was arrived at using quadrature method. The data on density of *Solanum carolinense* was recorded and subjected to further analysis.

RESULTS

Solanum carolinense (Family: Solanaceae) was observed Kambainallur Block in Dharmapuri District and Vadavalli Block in Coimbatore District of Tamil Nadu. *Solanum carolinense* is a native of Gulf States, and is distributed in temperate and tropical zones of North America, South America, Oceania, Europe and part of Asia. It is regarded as an environmental and ornamental weed, as well as a problematic and poisonous weed.

During the survey, relatively few plants of this invasive species were initially found near the electric power transformer adjacent to village garbage disposal area. Plants found were about one meter tall, armed with small spines. The erect stems possessed stellate hairs, which were greenish to purple in colour. Leaves were typically lance-ovate, lobed with spines on the midrib, with alternate veins.

The inflorescence was axillary racemes with some branching, bearing compact flowers. The flower was five lobed and the corolla was white to lilac or purple in colour (Plate 1.). The flower also had stellate pubescence externally and was glabrous inside. It contained five stamens filaments yellowish green in colour. The style of the flower was glabrous and greenish in colour. Stigma was dark in colour and the superior ovary was glandular, pubescent and whitish in colour. The calyx was deeply lobed, five in number and the calyx tube purple green in colour. The mature fruit was yellowish in colour and globular, and the immature fruit green in colour.



Plant with spiny leaf



Flowers



Unripened Fruits



Ripened Fruits



Cross section of Fruits



Seeds

Plate 1. Characteristics of *Solanum carolinense*

The fruit, a single berry, contained 120 to 240 seeds. Seeds were flattened, round with a peak yellowish to orange brown. Plants appeared to flower throughout the year with peak flowering from July to November (South West and North East Monsoon Seasons) in Tamil Nadu conditions. This phenomenon is in contrast to the flowering and fruit setting with *Solanum viarum* in Florida, which is from September through May (fall and winter seasons) due to favorable photoperiod and day/night temperatures (Mullahey *et al.* 1993).

Incidence of *Solanum carolinense* in Tamil Nadu

Out of 218 plants of *Solanum carolinense* found in eight districts of Tamil Nadu during the survey, Coimbatore recorded highest incidence, closely followed by Dindugal, Dharmapuri, Krishnagiri and Salem districts (Table 1). Coimbatore district recorded 31.6% of the total observed *Solanum carolinense* followed by 14.7, 13.8, 12.8 and 12.4 per cent, respectively in Dindugal, Dharmapuri, Salem and Krishnagiri districts (Figure 1).

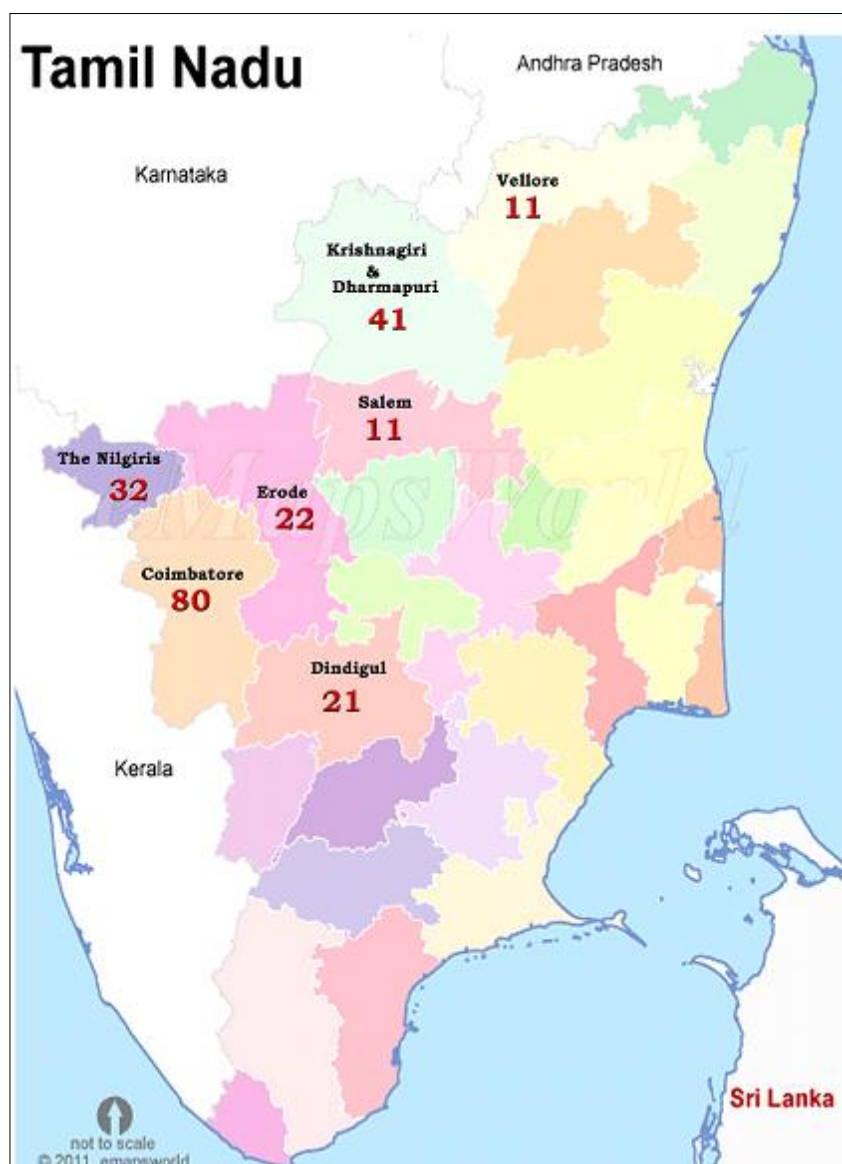


Figure 1. Map showing Districts of Tamin Nadu and incidences of *Solanum carolinense* recorded during the survey

In the Coimbatore District, about 69 plants was identified, in which Anamalai Block was recorded with higher number (40) of *Solanum carolinense* compared to other blocks within the Coimbatore District. In Dindugal District, about 32 plants were identified in Thoppampatti and Kodaikanal blocks. In Kodaikanal block of Dindugal district, 17 *Solanum carolinense* plants were recorded; and in the Udthagamandalam and Kotagiri blocks of Nilgiris District, 15 plants were found.

Table 1. Incidence of *Solanum carolinense* in different districts of Tamil Nadu

Districts	Name of the blocks	Village	Number of <i>Solanum carolinense</i> Plants
	Thondamuthur	Vadavalli	8
	Kinathukadavu	Mullupadi	3

Coimbatore	Pollachi north	Eripatti	4
		Santhegoundenpalayam	5
	Pollachi south	Kolarpatti	3
		S.ponnapuram	4
	Udumalaipettai	Udumalaipettai	2
Dindugal	Anamalai	Manakadavu	40
	Thoppampatti	Puliyampatti	3
		Adukkam	8
		Thandikudi	4
	Kodaikanal	Kumbaraiyur	10
		Kamanur	4
		Pachalur	3
Nilgiris	Udhagamandalam	Kaguchi	8
	Kotagiri	Kunjapanai	7
Salem		C.kalrayan, Therkkunadu	9
	Peddanaikenpalayam	P.kalrayan, Melnadu	8
		P.kalrayan, Keelnadu	8
	Omalur	Sikkampatti	3
Erode	Thalavadi	Thalavadi	6
Dharmapuri	Morapur	Kambatanallur	10
		Karimangalam	6
	Karimangalam	Mottur	4
		Kondrampatti	6
	Pallakodu	Ammanimallapuram	4
Krishnagiri		Kudimanahalli	8
	Kaveripattinam	Bannihalli	5
		Bargur	6
		Erumpatti	8
Vellore	Arcot	Arappakkam	6
		Poongodu	5
Total			218

Management of *Solanum carolinense*

As a response to the recording of *Solanum carolinense*, consideration was given to its eradication. Hand weeding is recommended, as and when an infestation is noticed in an area. Manual removal requires the whole plant along with roots to be dug out, collected and burnt. Post-emergence application of a selective herbicide, such as 2,4-D at 1.25 kg/Ha, provides effective control of *Solanum carolinense* in cereal-cropped fields. A sequential application of glyphosate at 1 kg/Ha provided very good control of this invasive weed in non-crop situation. In the USA, consistent and effective control of similar type of plant, *Solanum viarum* had been achieved with Milestone® (0.08 kg/Ha aminopyralid) applied at broadcast rates ≥ 0.08 kg/Ha (Ferrell *et al.* 2006) or as a spot treatment at 15-20 mL/9.46 L (Sellers *et al.* 2009).

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LONG TERM EFFECT OF HERBICIDES ON WEED SHIFT AND YIELD OF RICE-YELLOW SARSON SYSTEM IN THE LATERITIC SOIL OF WEST BENGAL, INDIA

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ABSTRACT

A field experiment was conducted on sandy loam soil at Sriniketan, Visva-Bharati University, Birbhum, West Bengal, India during 1999 to 2009 in medium land transplanted rice-yellow sarson cropping system to know the effect of continuous use of weed management practices on weed shift and yield. Continuous use of butachlor at 1.0 kg as pre-emergence + 2, 4-D (Na- Salt) at 0.4 kg as post emergence in rice paved the way for dominance of sedge like *Fimbristylis miliacea* and grass like *Digitaria sanguinalis* in rice under rice- yellow sarson cropping system. Rotational use of pretilachlor at 1.0 kg and butachlor at 1.0 kg as pre-emergence + 2, 4- D (Na-Salt) at 0.5 kg with organic matter lowered the menace of broadleaved as well as sedges, but not the grasses and registered higher yield of rice in 2009. Continuous or rotational use of pretilachlor or butachlor + 2, 4- D (Na- Salt) resulted in disappearance of *Hydrolea zeylanica* but *Cynodon dactylon* appeared as one of the dominant grassy weeds in 2009. Similarly continuous or rotational use of pendimethalin and isoproturon at 1.0 kg in yellow sarson lowered the density of *Digitaria sanguinalis* in 2009. *Cynodon dactylon* and *Croton bonplandianum* which were not present initially came into dominant group in the final year. Rotational use of isoproturon or pendimethalin along with FYM reduced the number of grasses and broadleaved weeds and registered higher yield of yellow sarson over other treatments.

Keywords: herbicides, long term effect, rice-yellow sarson cropping system, repeated and rotational use of herbicide, weed shift.

INTRODUCTION

Weed management in agricultural lands is rapidly shifting towards chemical method because of its several advantages. Herbicides are the most effective and economically acceptable means of weed management. Use of herbicide is rapidly increasing in the world including India. It has revolutionized the weed management in world agriculture. In the last three decades evolution of newer herbicide molecules provided wider choice for the farmer. But continuous use of the same herbicide year after year may lead to several problems out of which the shift in weed flora is the prominent one. Rice-Yellow sarson is one of the important cropping systems in eastern India including West Bengal. The nature and extent of weed flora in transplanted rice are influenced by the puddling, depth of water and other cultural practices. Again the weed flora composition and population dynamics are also regulated by use of selective herbicide for weed management. Very specific

herbicides with low dose are being recommended for effective weed management in transplanted rice as well as yellow sarson. Weed population dynamics under integrated nutrient management involving integrated use of organic and other sources of nutrients along with chemical fertilizer in different crops and cropping system has been another important areas of study. Under these circumstances it is desirable to know the effect of long term use of herbicides along with chemical fertilizer alone or in combination with organic matter on weed shift and productivity in rice yellow sarson cropping system.

MATERIALS AND METHODS

A field experiment was conducted on sandy loam soil at the Farm of the Institute of Agriculture, Visva-Bharati University, West Bengal, India during rainy season 1999 to winter season 2009-10 in medium land rice-yellow sarson cropping system to know the effect of continuous and rotational use of weed management treatments on weed shift and yield of rice-yellow sarson cropping system. The permanent experiment in rice-yellow sarson cropping sequence was initiated in 1999 and continued in the same layout with rotational use of herbicides. In rice 5 treatments comprising of farmer's practice i.e two hand weeding at 30 & 45 days after transplanting (DAT), continuous use of butachlor at 1.0 kg /ha as pre-emergence followed by 2,4-D (Na salt) at 0.5kg/ha + recommended dose of fertilizer (RDF) through chemical fertilizer @ 60:30:30 kg N, P₂O₅ and K₂O/ha , continuous use of butachlor at 1.0 kg /ha as pre-emergence followed by 2,4-D (Na salt) at 0.5kg/ha + 70% of RDF through chemical fertilizer + 10 t /ha green leaf manuring, rotational use of pretilachlor / butachlor at 1.0kg as pre-emergence followed by 2,4-D (Na salt) + 100% RDF through chemical fertilizer and rotational use of pretilachlor / butachlor at 1.0kg as pre-emergence followed by 2,4-D (Na salt) + 70% RDF through chemical fertilizer + 10 t /ha green leaf manuring were assigned in a randomized block design with three replications. In case of follow up crop of yellow sarson, ten treatments were taken dividing each plot of rice into two- one with 100% RDF only and another with 70% RDF and farm yard manure (FYM) at 6 ton/ha to observe the differential effect of fertilizer and FYM on yellow sarson. Recommended dose of fertilizer @ 80:4:40 N, P₂O₅ and K₂O kg/ha was applied in yellow sarson as per treatment. Green leaf manuring (with *Antigonon leptopus* — a creeping weed of Polygonaceae family) @ 10 t/ha was applied one month before final puddling operation of rice field and in yellow sarson, F.Y.M. @ 6 t/ha was applied as per treatments. Effect of continuous use of herbicides and herbicide-fertilizer/organic matter combination on weed shift and grain yield of paddy and yellow sarson was recorded in the initial and final year. The rice variety IR-36 was transplanted in rainy season at a common spacing of 20 × 15 cm by using 30-35 days aged seedlings. The yellow sarson variety B-9 was shown in winter season with row to row spacing of 30cm. The gross and net plot sizes were 6.5 × 5m and 3 × 5m respectively. The herbicides were applied by using knapsack sprayer with five hundred litres of spray volume /ha as per treatment at 2 days after transplanting/sowing (DAT/DAS). The species and category wise weed density and dry weight was recorded in quadrat of 50 × 50 cm at 60 DAT/ DAS in all the seasons.

RESULTS

Major weed flora

Rice

Major weed flora observed in the experimental field of rice during rainy season of 1999 to 2009 were *Digitaria sanguinalis*, *Cynodon dactylon*, *Panicum repens*, *Paspalum distichum* among grasses; *Hydrolea zeylanica*, *Ludwigia parviflora*, *Spilanthus acmella*, *Alternanthera sessilis*, *Lindernia crustacea* among broadleaved and *Fimbristylis miliacea*, *Cyperus iria* and *Cyperus difformis* among sedges. Other weeds present in lower density were *Eleusine indica*, *Dactyloctenium aegyptium*, *Setaria glauca*, *Echinochloa colonum*, *Eclipta prostrata*, *Lindernia ciliata*, *Ammania baccifera*, *Oldenlandia corymbosa* and *Commelina nudiflora*.

Yellow sarson

Major weed flora observed in the yellow sarson field during winter 1999-2000 to 2009-2010 were *Digitaria sanguinalis*, *Cynodon dactylon*, *Echinochloa colonum* among grasses; *Anagallis arvensis*, *Spergula arvensis*, *Chenopodium album*, *Gnaphalium purpureum*, *Polygonum plebeium* among broad-leaved weeds. Other weeds in lower density were *Eleusine indica*, *Alternanthera sessilis*, *Eclipta prostrata*, *Spilanthus acmella*, *Solanum nigrum* and *Ageratum conyzoides*.

Weed shift

Rice

To know the shift in weed flora due to continuous use of herbicides the data on species wise weed density along with category of weeds (grass, broadleaved and sedge) at 60 DAT have been presented in Table 1. In the initial year (rainy season 2009) the dominant grass, broadleaved and sedge in the experimental field were *Digitaria sanguinalis*, *Hydrolea zeylanica* and *Fimbristylis miliacea* respectively.

Table 1. Long term effect of herbicides on weed shift (density of major weed species) in rice under rice-yellow sarson cropping system.

Treatments Weed density (no./m²) at 60 DAT in rice in the initial year, 1999

ts (in rice)																		
	Grass				Broad-leaved								Sedge					
	Cd	E	D	Tot	A	C	E	H	L	L	M	O	S	Tot	C	C	F	Tota
		c	s	al	s	n	p	z	c	p	q	c	a	al	d	i	m	l
T ₁	0	1	2	3	1	0	1	34	1	5	2	1	9	54	1	1	7	9
T ₂	0	1	2	3	2	0	1	32	1	4	2	1	10	53	1	1	4	6

T ₃	0	2	2	4	2	0	1	31	2	5	1	1	$\frac{1}{1}$	54	1	1	6	8
T ₄	0	1	3	4	2	0	2	32	1	4	2	1	$\frac{1}{0}$	54	1	1	7	9
T ₅	0	1	2	3	2	0	1	31	1	4	2	1	$\frac{1}{0}$	52	1	1	8	10
Treatmen ts (in rice)	Final year 2009																	
	Grass				Broad-leaved								Sedge					
	Cd	E c	P s	D s	Tota l	A c	C n	L c	Lc r	L p	O c	P s	S a	Tot al	C i	F m	Tot al	
T ₁	35	1 2	5 2	2 0	119	0	4	4 4	4	9	4	5	2 4	94	8	1 2	20	
T ₂	32	3 6	8 0	1 9	167	0	4	0	0	6	0	4	6	20	0	8	8	
T ₃	60	6 0	5 2	2 4	196	4	6	0	0	8	0	0	6	24	0	1 0	10	
T ₄	72	8 4	3 2	1 4	202	2	5	0	0	4	4	2	1 0	27	0	0	0	
T ₅	120	4 4	6 8	1 7	249	4	6	0	0	5	5	3	9	32	0	0	0	

T₁: Farmer's practice- two hand weeding at 30 & 45 DAT; T₂: continuous use of butachlor at 1.0 kg /ha followed by 2,4-D (Na salt) at 0.5kg/ha + 100% RDF through chemical fertilizer; T₃: Continuous use of butachlor at 1.0 kg /ha followed by 2,4-D (Na salt) at 0.5kg/ha + 70% of RDF through chemical fertilizer + 10 t /ha green leaf manuring; T₄: Rotational use of pretilachlor / butachlor at 1.0kg followed by 2,4-D (Na salt) + 100% RDF through chemical fertilizer and T₅: Rotational use of pretilachlor / butachlor at 1.0kg followed by 2,4-D (Na salt) + 70% RDF through chemical fertilizer + 10 t /ha green leaf manuring

Cd-Cynodon dactylon, Ec-Echinochloa colonum, Ps-Paspalum scorbiculatum, Ds-Digitaria Sanguinalis, Ac- Ageratum conyzoides, Cn- Commelina nudiflora, Lc-Lindernia ciliata, Lcr-Lindernia crustacea, Lp-Ludwigia parviflora, Oc-Oldenlandia corymbosa, Ps-Phyllanthus simplex, Sa-Spilanthes acmella, Ci-Cyperus iria, Fm-Fimbristylis miliacea, As-Alternanthera sessilis, Ep- Eclipta prostrata, Hz- Hydrolea zeylanica, Mq- Marsilea quadrifolia, Cdf-Cyperus difformis

Continuous use of butachlor at 1.0kg as pre-emergence + 2,4-D (Na-salt) at 0.4 kg as post-emergence in rice though controlled broadleaved weeds but resulted in the

dominance of grasses and sedges in the final year (rainy season 2009). Rotational use of pretilachlor at 1.0kg and butachlor at 1.0kg as pre-emergence + 2,4-D (Na-salt) at 0.4 kg post-emergence lowered the menance of broad-leaved and sedge but not the grasses (Figure 1.). Effect of continuous use of butachlor or pretilachlor / butachlor rotation was found better in reducing broad-leaved and sedges when applied with fertilizer alone than with organic matter. Continuous use of butachlor or rotational use of pretilachlor / butachlor in addition to fertilizer or organic matter resulted in disappearance of *Hydrolea zeylanica* which initially was the most dominant broad-leaved weed. New appearance of *Cynodon dactylon* and *Paspalum* sp. and increasing density of *Digitaria sanguinalis* resulted in more dominance of grass in final year both in continuous use of butachlor and rotational use of pretilachlor / butachlor.

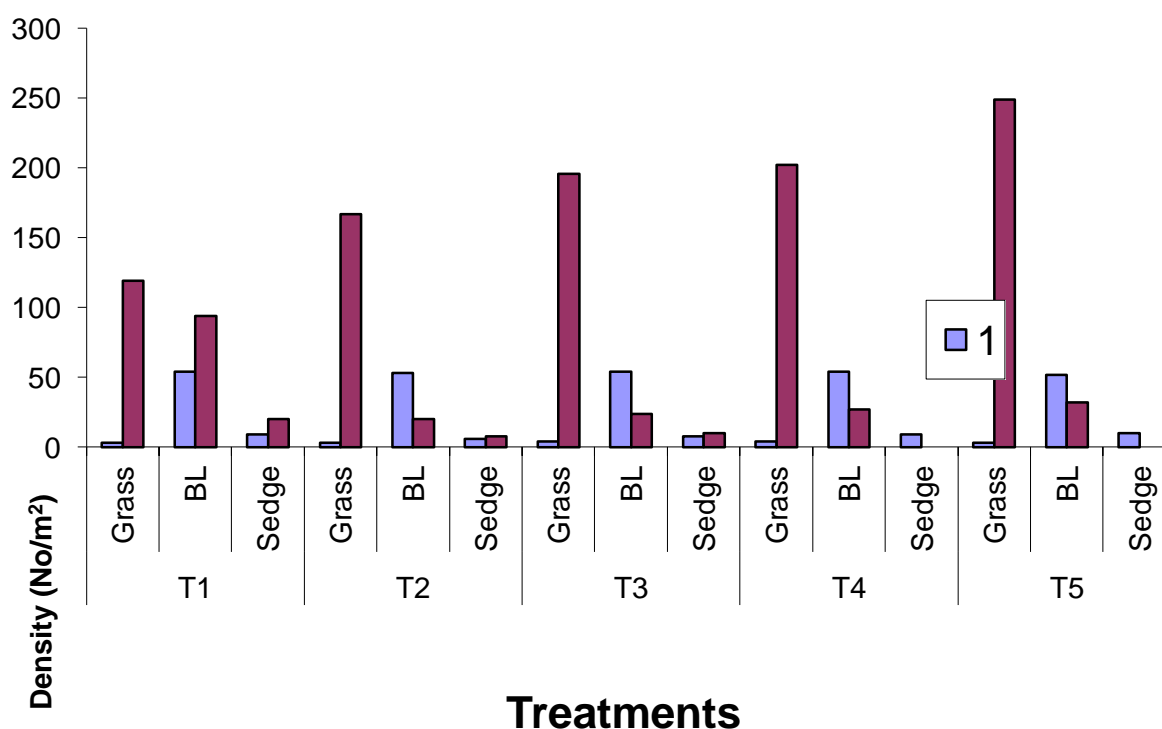


Fig. 1. Shift in weed flora in terms of density of weed category under different treatments of rice in initial rainy 1999 and final 2009

Yellow sarson

In the initial year the total number of grasses and broad-leaved weeds was uniform in all the treatments before application of herbicides. However use of pendimethalin along with fertilizer or FYM could not reduce the total grass population. But rotational use of isoproturon / pendimethalin reduced the total grass population to the tune of 7 – 21% in 2009-10 (Table 2.). However, the reduction in total grass population with the application of

fertilizer only was more than that of application with FYM. Out of the total grasses, *Echinochloa colonum*, which was initially absent, appeared in final year. Again *C. dactylon* which was initially present in very low density appeared as dominant in all the treatments except farmers practice. But the density of *Cynodon* in continuous use of pendimethalin was increased more where FYM was used along with fertilizer as compared to sole application of fertilizer. The number of *C. dactylon* under rotational use of isoproturon / pendimethalin was less as compared to continuous use of pendimethalin. Application FYM along with fertilizer in rotational use of isoproturon /pendimethalin resulted in the increase in number of *Cynodon dactylon* as compared to sole application of fertilizer (Table2.).

Table 2. Long term effect of herbicides on weed shift (density of major weed species) of yellow sarson under rice-yellow sarson cropping system.

Treatments (in yellow sarson)	Weed density (no./m ²) in yellow sarson (initial year 1999-2000)														
	Grass					Broad-leaved									
	Cd	Ds	Ec	Total	As	Aa	Ca	Cb	Gp	Pp	Sn	Sa	Spa	Ep	Total
T ₁	1	58	0	59	1	1	0	0	1	0	0	2	2	0	7
T ₂	1	54	0	55	1	1	0	0	2	0	0	2	2	0	8
T ₃	1	51	0	52	1	1	0	0	1	0	0	2	2	0	7
T ₄	1	58	0	59	1	1	0	0	1	0	0	1	3	0	7
T ₅	1	52	0	53	1	1	0	0	1	0	0	2	6	0	11
T ₆	1	55	0	56	0	1	0	0	1	0	0	1	2	0	5
T ₇	2	50	0	52	1	1	0	0	1	0	0	2	2	0	7
T ₈	1	52	0	53	0	1	0	0	1	0	0	3	2	0	7
T ₉	2	54	0	56	0	1	0	0	1	0	0	1	2	0	5
T ₁₀	1	54	0	55	0	1	0	0	1	0	0	1	1	0	4
Treatments (in yellow sarson)	(Final year 2009-10)														
	Grass					Broad-leaved									
	Cd	Ds	Ec	Total	As	Aa	Ca	Cb	Gp	Pp	Sn	Sa	Spa	Ep	Total
T ₁	26	28	3	57	2	0	9	13	3	7	2	0	4	5	45
T ₂	28	30	4	62	2	0	11	16	3	9	3	0	6	7	57

T ₃	40	13	1	54	3	0	5	17	0	6	1	0	3	1	36
T ₄	42	15	1	58	4	0	6	20	1	7	2	0	4	2	46
T ₅	39	9	4	52	4	0	8	16	2	9	2	0	5	2	48
T ₆	41	12	5	58	5	0	10	18	3	10	3	0	6	3	58
T ₇	29	11	3	43	2	0	5	10	2	4	2	0	4	0	29
T ₈	32	13	4	49	1	0	3	12	4	7	3	0	5	1	36
T ₉	30	12	2	44	1	0	4	9	3	4	1	0	3	2	27
T ₁₀	34	13	3	50	1	0	3	11	4	6	2	0	5	3	35

T₁-Farmers practice (one hand weeding) + 100 % RDF, T₂- Farmers practice + 70 % RDF through chemical fertilizer + FYM @ 6 t/ha, T₃ – Continuous use of pendimethalin at 1.0 kg/ha + 100% RDF, T₄- Continuous use of pendimethalin at 1.0 kg/ha + 70 % RDF through chemical fertilizer + FYM @ 6 t/ha, T₅ – Continuous use of pendimethalin at 1.0 kg/ha + 100% RDF, T₆ – Continuous use of pendimethalin at 1.0 kg/ha + 70 % RDF through chemical fertilizer + FYM @ 6 t/ha, T₇ – Rotational use of isoproturon/pendimethalin + 100 % RDF, T₈ – Rotational use of isoproturon/pendimethalin +70 % RDF through chemical fertilizer + FYM @ 6 t/ha, T₉ – Rotational use of isoproturon/pendimethalin + 100 % RDF, T₁₀ – Rotational use of isoproturon/pendimethalin +70 % RDF through chemical fertilizer + FYM @ 6 t/ha.

Cd-Cynodon dactylon, Ds-Digitaria sanguinalis, Ec-Echinochloa colonum, As-Alternanthera sessilis, Aa-Anagallis arvensis, Ca-Chenopodium album, Cb-Croton bonplandianum, Gp-Gnaphalium purpureum, Pp-Polygonum plebeium, Sn-Solanum nigrum, Sa-Spergula arvensis, Spa-Spilanthes acmella, Ep-Eclipta prostrata.

Similarly, continuous or rotational use of pendimethalin and isoproturon in yellow sarson lowered the density of *Digitaria* in 2009-10 to the tune of 74 -82%. However, the reduction in number was more where fertilizer was applied alone as compared to combined application of fertilizer & FYM. No sedge was observed in the yellow sarson. Initially the number of total broad-leaved weeds was less as compared to grasses. But with the progress of the experiment the higher number of total broad-leaved weeds was registered. Number of total broad-leaved weeds increased more in continuous use of pedimethalin than that of rotational use of isoproturon and pendimethalin. Again, the number of broad-leaved weeds was more where fertilizer was applied along with FYM than sole application irrespective of continuous or rotational use of pendimethalin / isoproturon (Table 2.).

Out of the total broad-leaved weeds *Chenopodium album*, *Croton bonplandianum*, *Polygonum plebeium* and *Solanum nigrum* were initially absent but with the progress of the experiment, they began to appear and *Croton bonplandianum* became the most dominant among the broad-leaved weeds followed by *Polygonum plebeium* and *Chenopodium album* in the final year (2009-10). On the other hand *Anagallis arvensis* and

Spergula arvensis which were initially present in low density were totally disappeared in the final year.

Yield of paddy

The data on paddy yield (kg/ha) for the year 1999 to 2009 along with overall mean are provided in Table 3. In the initial year there was no significant difference in paddy yield among the treatments.

Table 3. Long term effect of weed management practices on paddy yield in transplanted rice under rice-yellow sarson cropping system.

Treatment s	Grain yield of paddy (kg/ha)											Mean
	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	
T ₁	4933	3457	3567	3883	3600	3567	3833	3682	3992	4185	4188	3899
T ₂	4917	4020	3600	3600	3530	3475	3750	3575	3983	3933	4131	3865
T ₃	4283	3807	3383	3650	3470	3475	3775	3584	4075	4205	4183	3808
T ₄	4183	4133	3300	3883	3687	3550	3792	3615	4058	3911	4166	3843
T ₅	3967	3817	3467	3917	3783	3583	3853	3635	4142	4311	4313	3890
LSD (P=0.05)	N.S.	215	224	N.S.	N.S.	N.S.	NS	NS	NS	188	143	NS

T₁: Farmer's practice- two hand weeding at 30 & 45 DAT; T₂: continuous use of butachlor at 1.0 kg /ha followed by 2,4-D (Na salt) at 0.5kg/ha + 100% RDF through chemical fertilizer; T₃: Continuous use of butachlor at 1.0 kg /ha followed by 2,4-D (Na salt) at 0.5kg/ha + 70% of RDF through chemical fertilizer + 10 t /ha green leaf manuring; T₄: Rotational use of pretilachlor / butachlor at 1.0kg followed by 2,4-D (Na salt) + 100% RDF through chemical fertilizer and T₅: Rotational use of pretilachlor / butachlor at 1.0kg followed by 2,4-D (Na salt) + 70% RDF through chemical fertilizer + 10 t /ha green leaf manuring

In the second year butachlor at 1.0 kg followed by 2,4-D at 0.4 kg /ha + 100 RDF through chemical fertilizer registered significantly higher yield as compared to other treatments where organic matter was applied. In the subsequent years the grain yield of paddy was slightly higher where butachlor at 1.0 kg followed by 2, 4-D at 0.4 kg /ha was applied along with organic matter than with chemical fertilizer alone. It is clear that use of herbicide butachlor or pretilachlor resulted in paddy yield similar to that of farmers practice i.e two

hand weedings. Use of fertilizer along with organic matter favoured slightly for higher yield than the use of fertilizer alone even after eleventh year of rice crop (Table 3). The yield of paddy was higher in the plots where pretilachlor and butachlor was used rotationally as compared to continuous use of butachlor. This indicates that the continuous use of same herbicide slightly lowered the paddy yield owing to lack of control of weeds particularly grasses and sedges.

Yield of yellow sarson

The seed yield of yellow sarson did not vary significantly among the treatments in initial year. In the third, fourth and fifth crop of yellow sarson the yield was significantly reduced in the plots where pendimethalin was applied. In the subsequent years the yield was slightly higher in the plots where FYM was applied along with fertilizer and where herbicides were used rotationally (Table 4.). Rotational use of isoproturon or pendimethalin along with FYM registered higher yield of yellow sarson over other treatments in the later years.

Table 4. Long term effect of weed management practices on seed yield of yellow sarson under rice yellow sarson cropping system.

Seed yield of yellow sarson (kg/ha)

Treatments	1999-2000	2000-01	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09	2009-10	Mean
T ₁	1147	1069	1017	977	982	1208	984	1227	1061	1088	1097	1078
T ₂	1163	1096	1025	1000	1052	1233	1067	1255	1110	1117	1131	1114
T ₃	1233	1189	300	700	387	1167	1017	1174	1072	1096	1101	949
T ₄	1177	1037	425	647	432	1142	1113	1182	1152	1159	1178	968
T ₅	1220	1142	317	670	490	1133	1055	1170	1085	1100	1115	954
T ₆	1197	1094	458	683	558	1142	1123	1185	1175	1184	1223	1002
T ₇	1133	1139	942	733	965	1225	1088	1223	1094	1106	1120	1070

T ₈	107 3	108 9	925	750	997	12 08	11 30	1227	1164	1194	120 7	108 8
T ₉	115 7	109 2	933	717	993	12 33	10 95	1231	1097	1112	118 7	107 7
T ₁₀	109 3	106 3	933	833	104 2	12 17	11 48	1244	1187	1203	124 7	111 0
LSD (P=0.05)	N.S.	NS	163	113	171	NS	NS	NS	NS	NS	NS	

T₁-Farmers practice (one hand weeding) + 100 % RDF, T₂- Farmers practice + 70 % RDF through chemical fertilizer + FYM @ 6 t/ha, T₃ – Continuous use of pendimethalin at 1.0 kg/ha + 100% RDF, T₄- Continuous use of pendimethalin at 1.0 kg/ha + 70 % RDF through chemical fertilizer + FYM @ 6 t/ha, T₅ – Continuous use of pendimethalin at 1.0 kg/ha + 100% RDF, T₆ – Continuous use of pendimethalin at 1.0 kg/ha + 70 % RDF through chemical fertilizer + FYM @ 6 t/ha, T₇ – Rotational use of isoproturon/pendimethalin + 100 % RDF, T₈ – Rotational use of isoproturon/pendimethalin +70 % RDF through chemical fertilizer + FYM @ 6 t/ha, T₉ – Rotational use of isoproturon/pendimethalin + 100 % RDF, T₁₀ – Rotational use of isoproturon/pendimethalin +70 % RDF through chemical fertilizer + FYM @ 6 t/ha.

DISCUSSION

The experimental results clearly indicates that among all the categories of weeds broadleaved was killed effectively by application of butachlor along with 2,4-D which is basically a broadleaved killer. The combined use of these herbicides was not effective in managing all the sedge species which ultimately resulted in the dominance of sedge and grassy weeds. Thus continuous use of butachlor at 1.0kg as pre-emergence + 2,4-D (Na-salt) at 0.4 kg as post-emergence in rice paved the way for dominance of grasses and sedges in the final year (rainy season 2009). Similar results were also reported by Anonymous (2006) and Prasad *et al.* (2008).

Again rotational use of pretilachlor and butachlor reduced the sedge population in the subsequent years indicating relatively more effectiveness of pretilachlor over butachlor in controlling sedge weeds in rice as also reported by Rajkhowa *et al.* (2001) and Ramachandra Prasad *et al.* (2008). Use of fertilizer along with organic matter favoured slightly for higher yield than the use of fertilizer alone even after eleventh year of rice crop (Table 3.) indicating cumulative effect of organic matter on paddy yield in the latter years. The results were in conformity with that of Ramachandra Prasad *et al.* (2008).

The yield of yellow sarson in the third, fourth and fifth years was significantly reduced in the plots where pendimethalin was applied. This was due to phytotoxicity caused by enhanced activity of pendimethalin as irrigation was applied immediately after application of the herbicide. Soil moisture plays an important role in the activity of this herbicide.

ACKNOWLEDGMENTS

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CHARACTERIZATION OF THE REPRODUCTIVE BEHAVIOUR AND INVASIVE POTENTIAL OF PARTHENIUM WEED IN AUSTRALIA

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ABSTRACT

Genetic and environmental and reproductive factors can play a vital role in the invasion success of plant species. In fact, recent studies indicate that plant reproductive systems are an important determinant of invasion success. To test these hypotheses, this study used two independent introductions of *Parthenium hysterophorus* L. (Asteraceae), which differ in invasion success post-introduction into Australia. Using individuals from both Clermont and Toogoolawah populations in this study, we examined some significant differences in their pattern of morphological characters, the floral structures and the reproductive behaviour of the two different populations of parthenium weed in Australia and assessed if differences identified in these populations correlate with invasiveness.

Key words: Invasive Species, Parthenium weed, Reproductive biology, Self-compatibility

INTRODUCTION

One of the most disturbing aspects of globalization is the movement of exotic plant species into virtually every ecosystem on earth (McNeely 2001; Vitousek 1997). Global trade has increased the opportunities for organisms to be spread widely and repeatedly introduced to new areas. Australia is facing weed invasions from within as well as from overseas. Every year new alien weeds are introduced into the country, and some that are already naturalized (including parthenium weed) extend their range to other parts of Australia (AWS 2006). When species are transported across biogeographic barriers to dispersal, it may become invasive, endangering the native species in the invaded ecosystem, undermining agriculture, threatening human and animal health and/or creating other unwanted and often irreversible effects on human life.

Both genetic and ecological factors are thought to play a vital role in the invasion success of plant species (Saki *et al.* 2001). Furthermore, recent studies suggest that reproductive systems are important determinants of genetic variation in species, which in turn may have impacts on the invasiveness of a species. However, little empirical evidence is currently available to support these hypotheses. As a case study, we have used *Parthenium hysterophorus* L. (Asteraceae), commonly known as parthenium weed, an invasive plant and agricultural weed in eastern Australia, to test these hypotheses. Parthenium weed is considered as a major weed of grazing land in central Queensland, where it significantly reduces the productivity of pastures (Navie *et al.* 1996). This species was first reported in the Toogoolawah district in south-eastern Queensland in 1956 and it has been suggested that this introduction occurred via machinery during the World War-II (Parsons and Cuthbertson- 1992). A second introduction is thought to have occurred in contaminated pasture seed lots in the Clermont district in central Queensland in 1958. The history

of spread of the weed from these two introductions is quite different. The Toogoolawah population seems to be much less invasive, with its population size remaining small and isolated, while the Clermont population has spread to cover many millions of hectares in central Queensland.

It should not be assumed that all populations of an introduced species will demonstrate invasiveness, since the propensity for invasiveness may be a result of genetic factors as well as ecological factors (Sakai *et al.* 2001). There may be different biological characters (especially growth and morphological attributes, reproductive behaviour and competitiveness) that may make one genetically distinct population of a weed more invasive than another. Thus, the present study was undertaken in order to examine whether various morphological, physiological and reproductive characters may have played a crucial role in the process of invasion.

MATERIALS AND METHODS

Seeds of the Clermont population (Central Queensland) and Toogoolawah population (South-east Queensland) of parthenium weed were obtained from the UQ seed collection held at the School of Agriculture and Food Sciences (SAFS) in Brisbane, Queensland. The seeds were germinated and 25 replicate seedlings from each population were individually grown in pots (25 cm diameter) in a temperature controlled glasshouse (28 ± 5 / 18 ± 2 °C: day/night). Data were then recorded for several different phenological characters. To investigate any differences in the floral morphology, five flowers from each replicate plant were collected and photographed using a Nikon SMZ-D AFX-11A photo microscopic system with FX-35 camera and their floral structures measured using Image Tool for Windows version 3.0, an image analysis software product developed by the University of Texas. In order to determine the reproductive behaviour of the plants, with respect to self-compatibility, five flowers from each plant were enclosed in small glassine bags. At the time of seed physiological maturity, the glassine bags containing flowers were collected and the seeds produced removed from the flowers. The bagged seeds were x-rayed to determine the percentage of seed filled in each population, and were compared with non-bagged flowers. The total percentage of filled seeds in each population was used as an indicator of self-compatibility.

Statistical Analysis

The data sets were subjected to two sample T-tests and one way ANOVA using the Minitab statistical package to determine if any of the measured parameters differed statistically between the Toogoolawah and Clermont populations.

RESULTS

Growth Patterns

The results clearly indicated that both the Clermont and Toogoolawah populations exhibited very similar growth patterns during the early rosette stage. However, as time progressed, the plants belonging to the Clermont population showed more vigorous growth attributes. The Clermont plants had produced significantly more leaves after both five and ten weeks of growth ($P < 0.001$). Both populations were also found to be significantly different from each other in some other morphological characters, such as average length of primary branch ($P = 0.003$) and height of

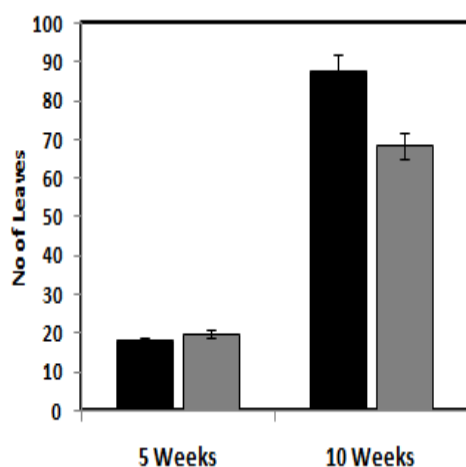
plant ($P < 0.001$). It is also evident from the results that seeds produced by the Clermont population were statistically larger than those produced by the Toogoolawah population. Seeds from both the Toogoolawah and Clermont populations reached greater than 90% germination in 3 weeks under ideal conditions (ca. 20-25 °C and 12/12 day/night). However, seeds from the Clermont biotype had an increased rate of germination (Figure 1).

Floral Morphology

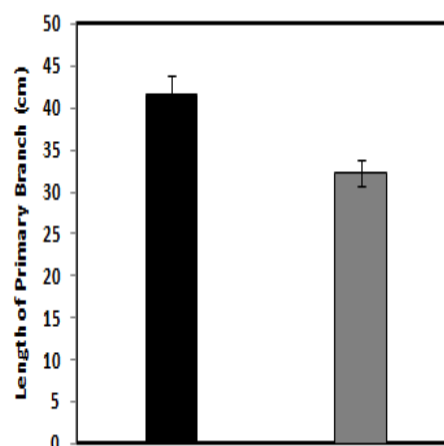
The morphology of floral structures plays a vital role in the pollination biology of plant species. It is clear from the data analysis that flowers produced by plants of the Toogoolawah population were quite different in structure when compared with flowers produced by plants of the Clermont population. In particular, the flowers of Clermont plants had significantly longer stamens and involucre bracts than those of Toogoolawah plants ($P < 0.001$). It was also apparent that Toogoolawah plants initiated their first flowers earlier than plants of the Clermont biotype (Figure 2).

Self-compatibility

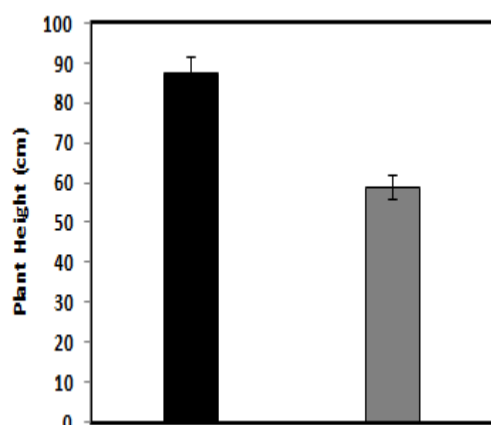
The results of this experiment indicate that the two populations of parthenium weed have significantly different levels of self-compatibility. In the control treatments, both the Clermont and Toogoolawah biotypes produced above 80% filled seeds. However, in the treatment with glassine bagged flowers, 73% of seeds produced by the Toogoolawah plants were filled compared to only 28% of seeds produced by the Clermont plants.



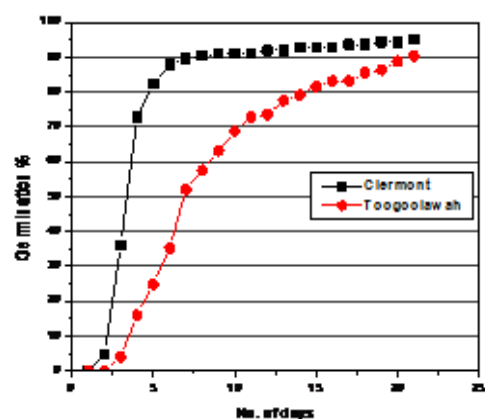
a)



b)

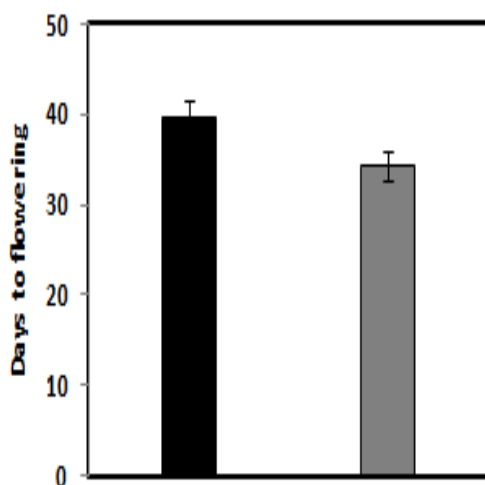


c)

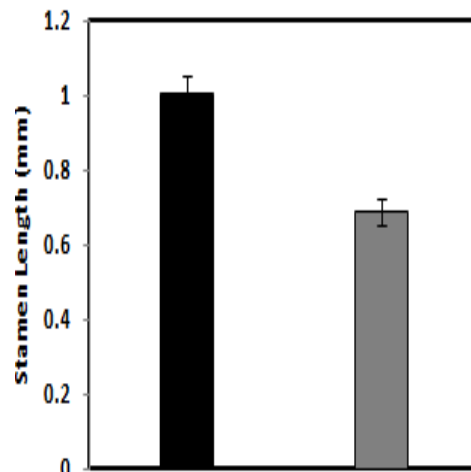


d)

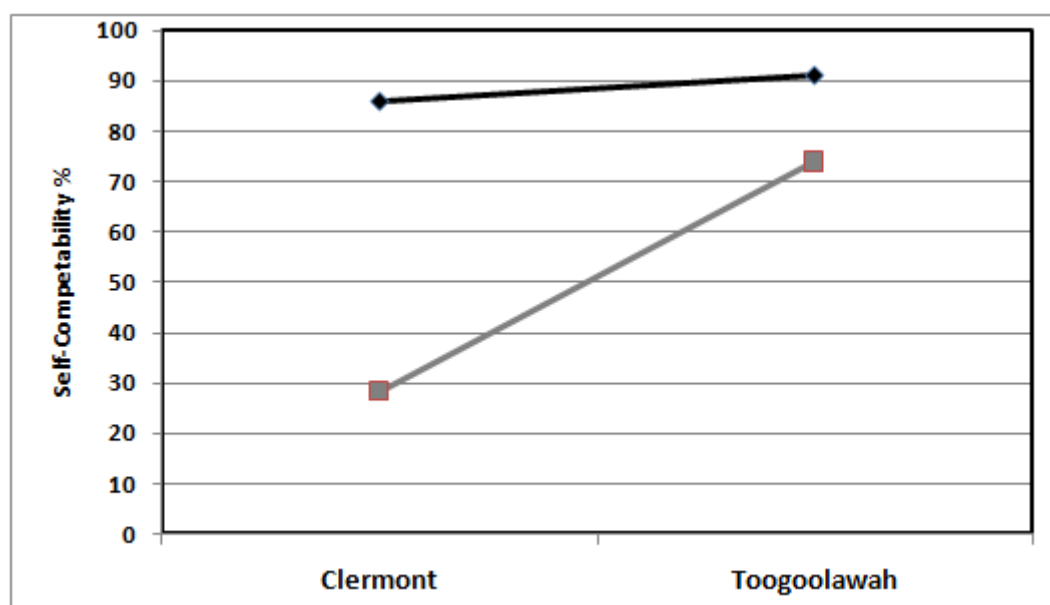
Figure 1. Mean values of several growth and germination parameters recorded from two populations of parthenium weed: a) number of leaves; b) average length of primary branch; c) Plant height d) germination rate. Values are the means of 25 replicates. Clermont and Toogoolawah.



a)



b)



c)

Figure 2. Mean values of several reproductive parameters recorded from two populations of parthenium weed: a) days to flowering b) stamen length c) self-compatibility levels. Values are the means of 25 replicates. Clermont and Toogoolawah populations.

DISCUSSION

Overall the results presented here show clear differences in morphological characters, seed physiology and reproductive systems between the two biotypes. While it would be nice to speculate which of these differences may be linked to increased invasiveness of the Clermont biotype, given that the biotypes differ in most attributes examined this is not possible. Instead differences between the biotypes will be discussed and related to the current literature on biological invasions.

The results recorded for various growth attributes showed that plants of the Clermont population displayed faster growth when compared to plants of the Toogoolawah population, especially after the bolting (i.e. stem elongation) stage of their life cycles. This difference is most probably due to different level of metabolism at different life stages or to the expression of genetic differences.

After the bolting stage of their life cycles, the two populations exhibited differential priorities for their further development. Plants of the Clermont population required a much shorter period of time to initiate stem elongation than plants of the Toogoolawah population, and also produced a larger number of leaves and branches. This may be considered to be a useful competitive trait, especially when parthenium plants are becoming established in a new area or during their subsequent secondary spread. Invasive plants with a taller stature, larger leaves and branches, and a faster growth rate would have an architecture which may enhance their success when they are exposed to competition with other species in the field.

It is also apparent from the results that the two populations of parthenium weed in Queensland are employing comparatively different seed production strategies. Plants of the Clermont population produced much larger seeds along with accessories in comparison with Toogoolawah. Pandey and Dubey (1988) have analogous observations regarding variations in seed size. The larger seeds of the Clermont biotype could be a factor facilitating short to long dispersals by water, wind and other agencies. The seeds of both populations showed a relatively high level of germination (i.e. above 90%) under ideal conditions (ca. 20-25 °C and 12/12 day/night) for 21 days. However, seeds of the Clermont population proved more opportunistic by achieved 50% germination in only a few days while seeds of the Toogoolawah population took more than a week to complete 50% germination. This opportunistic and rapid germination behaviour may be one reason why the Clermont population produces such large stands of plants following significant rainfall events in Central Queensland. Such a rapid germination rate may be an important contributing factor for seedling establishment and provide a competitive advantage (Baker, 1972). The germination behavior of the two populations may be due to different dormancy mechanisms inherited in them (Dixon *et al.* 1995). It may also depend upon several climatic factors, including the pattern of rainfall and the timings of subsequent germination events at a site (Coffin and Lauenroth 1989).

Keeping in mind the importance of floral morphology in the pollination biology of plant species, the reproductive structures of the Toogoolawah population were found to be reduced in size when compared to the Clermont population. The larger and broader floral structures of the Clermont plants may result in increased exposure and increased pollen capture from the surrounding plants. Such a reproductive strategy would likely favor cross-pollination, while the reverse is true for the Toogoolawah plants. Gupta and Chandra (1991) considered parthenium weed to be entomophilous or at most amphiphilous. The outcomes of the present research suggest that parthenium weed is not an obligate out-crosser, but it is capable of both pollination types, and that the level of self-compatibility varies from population to population. It was found to be as high as 73% in the Toogoolawah population and as low as 30% in Clermont population. A reasonable amount of self-compatibility is crucial to produce enough seeds during the secondary spread of parthenium weed into new locations. However, out-crossing is also important for maintaining genetic diversity in populations, which are then able to adapt to a wider range conditions and facilitate invasion.

Based on the very prominent differences observed in the physiology, morphology and reproductive systems of these two populations, future studies are planned to compare the competitiveness and genetic diversity of the Clermont and Toogoolawah populations. Furthermore, we will examine which traits are associated with invasiveness in the Clermont biotype, using crosses to introgress particular traits from the Clermont biotype into the Toogoolawah biotype and assessing their competitive ability in comparison to parental biotypes.

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WEED GROWTH AND GRAIN YIELD IN WHEAT AND RICE AS AFFECTED BY TILLAGE SYSTEM AND WEED MANAGEMENT PRACTICES IN RICE-WHEAT CROPPING SYSTEM IN BANGLADESH

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ABSTRACT

Rice-wheat (RW) cropping system is an important cropping system in Bangladesh. Crop production on permanent raised beds (PRB) with straw retention is expanding worldwide as a way to increase system productivity and weed management. A 2-year study was conducted at the Regional Wheat Research Centre, Rajshahi to compare the effects of tillage system and straw retention (SR) e.g. 30% SR of all crops + permanent raised beds (PRB), 0% SR + PRB, 30% SR + conventional tillage practice (CTP) and 0% SR + CTP in a intensified RW systems. The effect of herbicides was also evaluated for control. Bed planting led to higher grain yield both in rice and wheat than conventional planting. The population and dry weight of weeds were less in bed planting than in conventional planting. In both years the major infested weeds in wheat were: *Chenopodium album*, *Cynodon dactylon*, *Cyperus rotundus* and *Lolium* spp. In rice the major weeds were *C. dactylon*, *C. rotundus* and *Cyperus iria*. In wheat, Linture 70WG @ 375 g/ha at two leaves stage (10-15 days after seeding (DAS) was most effective in arresting weed growth as compared to 2, 4-D Amine @ 1.5 L/ha at four leaf stage (20-25 DAS) and was at par with hand weeding (30-35 DAS). Net profit was also the highest (17320 Tk/ha) in Linture 70 WG treated plots and the lowest (14680 Tk/ha) in hand weeded plots.

Keywords: Rice-wheat, Tillage options, Weeds, Weedicides, Lintur and Yield+

INTRODUCTION

Rice-wheat (RW) cropping systems are critical to food security of increasing population in Bangladesh. However, the sustainability of RW systems is threatened by productivity decline due to weed dynamics. Crop production on Permanent Raised Beds (PRB) with straw retention is expanding worldwide as a way to increase system productivity and weed management. Wheat is the second leading cereal crops in Bangladesh and is grown more or less all over Bangladesh as sole or intercrop. But the average yield of wheat is relatively low in contrast to the potential yields obtained in research fields. Wheat yield is severely reduced due to broad spectrum weed flora in different areas. Wheat is grown in winter when low temperatures favor germination and growth of important weeds like *Chenopodium* sp. (Hirano *et.al.*1991). Others have observed that broadleaf weeds were predominant in wheat fields (Mamun and Salim, 1989). Unchecked weed growth reduces crop yield up to 57% (Singh *et. al.*, 1997). Field experiments with wheat and herbicides in Bangladesh showed that weed populations are usually greater with minimum tillage and unweeded conditions (Quddus, 1989). Herbicidal weed control is well established in other wheat growing countries but in Bangladesh farmers mainly depend on manual weeding. Under the changing socio-economic condition workers are not available to undertake tedious agricultural operations. Grain yield of wheat can be achieved through proper weed management. Application of herbicides may be effective for proper weed management to boost the yield of wheat. The present investigation seeks to find alternate methods for weed control through herbicides without sacrificing yield.

MATERIALS AND METHODS

The investigation was carried out at the Regional Wheat Research Center Farm, Shyampur, Rajshahi, during winter season of 2004-2005 using wheat var. Shatabdi. There were twelve treatment combinations, rate and application timing given below:

<u>Treatments</u>	<u>Rate</u>	<u>Application timing</u>
-------------------	-------------	---------------------------

- | | | |
|-----------------|-------------------------|-------------------------------------|
| T ₁ | Lintur 70 WG- 250 g/ha | at two leaves stage.(10 - 15 DAS) |
| T ₂ | Lintur 70 WG- 300 g/ha | at two leaves stage. (10 - 15 DAS) |
| T ₃ | Lintur 70 WG- 375 g/ha | at two leaves stage. (10 - 15 DAS) |
| T ₄ | Lintur 70 WG- 250 g/ha | at four leaves stage. (20 - 25 DAS) |
| T ₅ | Lintur 70 WG- 300 g/ha | at four leaves stage. (20 - 25 DAS) |
| T ₆ | Lintur 70 WG- 375 g/ha | at four leaves stage. (30-35 DAS) |
| T ₇ | Lintur 70 WG- 250 g/ha | at six leaves stage. (30-35 DAS) |
| T ₈ | Lintur 70 WG- 300 g/ha | at six leaves stage. (30-35 DAS) |
| T ₉ | Lintur 70 WG- 375 g/ha | at six leaves stage. (30-35 DAS) |
| T ₁₀ | 2 - 4, D Amine 1.5 L/ha | at four leaves stage.(20- 25 DAS) |
| T ₁₁ | Hand weeding. | - One operation (30-35 DAS) |

T₁₂ = Control (No weeding).

A recommended dose of nitrogen (100 kg/ha) from urea, phosphorus (60 kg/ha) from triple super phosphate, potassium (40 kg/ha) from muriate of potash and sulphur (20 kg/ha) from gypsum were used. The entire amount of phosphorus, potassium and sulphur and 2/3rd of nitrogen were applied at the time of final land preparation. The remaining nitrogen was applied at CRI stage after 1st irrigation as a top dressing. Hand weeding was done after 1st irrigation. The experiment was laid out in RCB design with three replications. The unit plot size was 5m X 3m. Seeds were sown on 2 December, 2004. Three irrigations were applied at CRI, maximum tillering stage and grain filling stage. The various species of weed were counted in a 1m² quadrat at post emergence seedling. In the same area total numbers of various weeds were collected after 45 DAS and at harvest, and oven-dried weight of weeds were recorded. Data on yield and yield contributing characters were collected from randomly selected 1m² demarcation places and statistically analysed. Weed control efficiency was calculated as:

$$\text{WCE (\%)} = \frac{A-B}{A} \times 100$$

where A and B are the dry matter weight (g/m²) of weeds of control and treatments respectively.

Method of dry matter determination

The number of weeds per m² was calculated from 3 samples from the same plot. The weed species were separated into three categories, packed and labelled separately. Samples were oven-dried for 24 hours at 105^oC. Dry weight of the weed species were measured by electric balance at normal room temperature. Grain yield was recorded from five representative samples of 1 m² area per plot. Yield component data were recorded from 10 plants/plot. Data were analyzed following standard statistical procedure and means separated by DMRT.

RESULT AND DISCUSSION

Effect on weeds of different treatment

The minimum weed population and dry weight were recorded in raised beds with straw management systems, which was significantly less than in conventional beds without straw management treatments (Fig. 1). This might be due to straw management which suppressed the weed infestation. Similar results were reported by Verma and Sirvastava (1998).

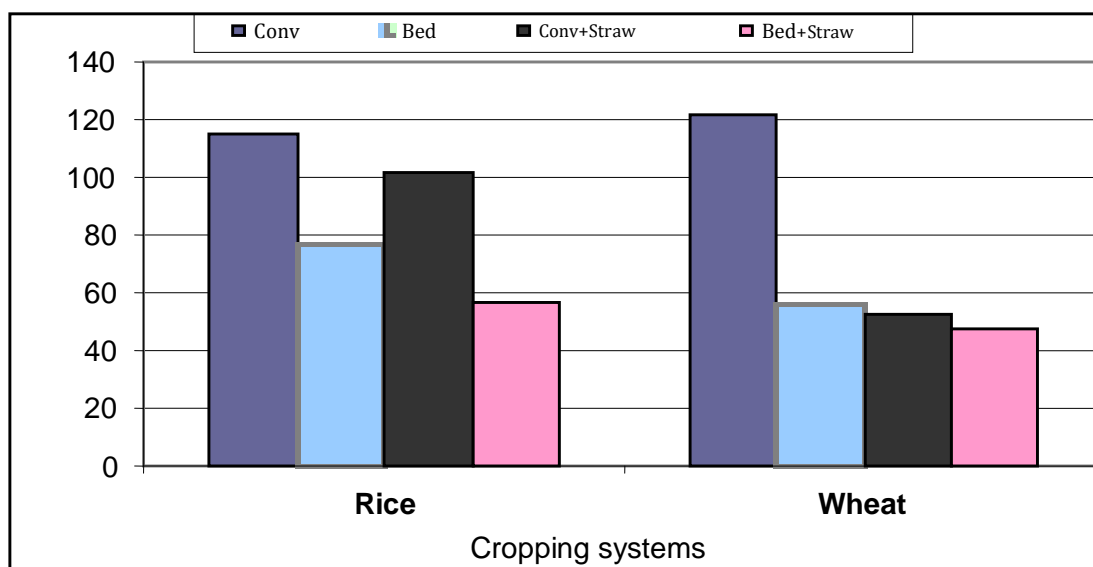


Figure 1. Effect of tillage options and straw management on weed dry weight.

The four major weed species in the wheat field were *Chenopodium album*, *Cynodon dactylon*, *Cyperus rotundus* and *Echinochloa crusgalli* (Table 1), the same weed species reported by Balyan (1999) in wheat fields. *Chenopodium album* and *C. rotundus* were dominant in all treatments. Weed population and biomass production of weeds were greatly influenced by different weedicides and times of application. All weedicide treatments significantly reduced weed population, resulting in lower dry biomass as compared to the control treatment, for all weed species. Significantly the lowest number of weeds/m² and dry biomass yield were recorded with Lintur 70 WG- 375 g/ha application at two leaf stage (10 - 15 DAS) followed by one hand weeding at 25 DAS. 2, 4 D Amine and Lintur 70 WG- 300 g/ha at two leaf stage (10 - 15 DAS) also reduced the weed population and dry matter production compared to control but not as much as Lintur 70 WG- 375 g/ha at two leaf stage (10 - 15 DAS) and hand weeding. Lintur 70 WG- 375 g/ha application at two leaf stage controlled all types of weeds but was more effective on broadleaf species.

Table 1: Number and dry weight of different weed species/m² as affected by weed control methods.

Treatments	Rate of application	<i>Chenopodium album</i>		<i>Cynodon dactylon</i>		<i>Cyperus rotundus</i>		<i>Echinochloa crusgalli</i>		Grain yield (t/ha)
		No.	Dry weight (g)	No.	Dry weight (g)	No.	Dry weight (g)	No.	Dry weight (g)	
Lintur 70 WG	250 g/ha	13	0.66	21	4.55	28	2.96	18	1.62	2.70de
Lintur 70 WG	300 g/ha	22	1.12	22	4.67	24	2.62	32	2.88	2.80cd
Lintur 70 WG	375 g/ha	4	0.20	12	2.55	20	2.01	19	1.90	3.40a
Lintur 70 WG	250 g/ha	18	0.92	18	3.82	32	3.49	20	1.92	3.13ab

Lintur 70 WG	300 g/ha	18	0.92	19	3.88	29	3.36	22	1.98	3.40a
Lintur 70 WG	375 g/ha	20	1.02	27	5.73	23	2.58	22	2.00	2.96bc
Lintur 70WG	250 g/ha	32	1.63	28	5.92	29	3.38	21	2.05	3.14ab
Lintur 70 WG	300 g/ha	23	1.17	20	4.36	16	1.72	33	2.94	2.80cd
Lintur 70 WG	375 g/ha	20	1.02	18	3.91	36	3.92	24	2.18	2.86cd
2,4 D Amine	1.5 L/ha	22	1.12	11	2.48	15	1.63	26	2.35	3.18ab
Hand weeding	One operation	23	1.17	4	0.86	13	1.42	4	0.36	3.30a
Control	No weeding	23	11.78	12	25.44	31	3.41	26	2.41	2.40e
		1		0						

In a column, means followed by common letters do not differ significantly at 1% level of DMRT

Effect on weeds

The herbicide treatments in general were found effective in killing the different weeds and reducing their dry matter production. The dry matter weight of weeds significantly decreased with one hand weeding (45 DAS) and application of Lintur @ 375 g/ha, as compared to dry matter wt. before spray (45 DAS) and the control. 2, 4 D Amine and Lintur @ 300 g/ha also decreased the dry matter weight compared to the control but not compared with hand weeding and Lintur 70 WG @ 375 g/ha, due to lower effectiveness on weeds. Further the weed dry matter production increased significantly with increased time due to decreased effectiveness of the herbicides in all treatments. The weed control efficiency of hand weeding was very high, 83.4% and 81.7% at 45 DAS and at harvest, comparable to Lintur @ 375 g/ha as compared to control and other treatments. The maximum weed population and dry weight per unit area were recorded in control treatments (no weeding) and the lowest in Lintur 70 WG-375 g/ha at two leaf stage, followed by Lintur WG 300 g/ha at four leaf stage and hand weeding (Table 2). Similar trends were found by Singh and Singh (1992). Weeds are less affected by Lintur 70 WG @ 250 g/ha at 6 leaf stage. Overall performance, Lintur 70 WG @ 375 g/ha at two leaf stage would be as good as controlling weeds by hand. Similar trend was found in dry weight of weeds, and similar results observed by Sharma *et.al.* (1999).

Table 2: Weed dry matter production (g/m²) and control efficiency (%) at 25 DAS and different time after spray.

Treatments	Rate of application	Before spray (g/m ²)	After spray(g/m ²)		Weed control efficiency(%)	
			45 DAS	At harvest	45 DAS	At harvest
Lintur 70 WG-	250 g/ha	30.45	10.2	13.90	66.5	54.3
Lintur 70 WG	300 g/ha	34.36	11.27	14.30	67.2	58.3
Lintur 70 WG	375 g/ha	38.70	6.61	7.21	82.9	81.3
Lintur 70 WG	250 g/ha	31.32	10.15	12.30	67.5	60.7

Lintur 70 WG	300 g/ha	30.20	10.14	11.35	66.4	62.4
Lintur 70 WG	375 g/ha	34.32	11.33	13.21	66.9	61.5
Lintur 70 WG	250 g/ha	31.20	12.98	14.23	58.3	54.4
Lintur 70 WG	300 g/ha	25.26	10.19	11.65	59.6	53.8
Lintur 70 WG	375 g/ha	26.65	11.03	12.98	58.6	51.2
2 - 4, D Amine	1.5 L/ha	34.20	7.58	8.97	77.8	73.7
Hand weeding.	One operation	30.25	3.81	5.51	83.4	81.7
Control	No weeding	31.23	43.04	51.32	-	-

Effect of straw retention and PRB on weeds

Weed infestation in each crop was reduced greatly by straw retention, especially in narrow leaf weeds in both crops and broadleaf weeds in wheat (Table 3). There was a consistent trend for greater reduction in weed population with 30% SR. Straw covering the soil surface reduced both weed seed germination and seedling growth. Crop residue retention can suppress weeds (Kumar and Goh 2000) by restricting solar radiation reaching the soil surface, by allelopathy and by reducing initial N availability through temporary immobilization. In addition to influencing germination and growth, straw retention and reduced tillage also influence the efficiency of soil-applied pre-emergence herbicides. In the absence of straw, there was a consistent trend for fewer weeds in PRB than conventional. The biggest effects were in wheat, where weed counts on PRB were about 50% of those in conventional beds for all three types of weeds.

Table 3. Yield losses (%) of wheat and rice as influenced by straw retention at different densities of weeds

Treatments	Wheat			% Yield loss	Rice			% Yield loss
	NL	BL	Sedge		NL	BL	Sedge	
0% SR+PRB	14	43	12	11	27	2	15	14
30% SR+PRB	7	13	5	3	11	0	8	6
0% SR+Conv	25	78	21	22	87	6	27	32
30% SR+Conv	11	23	9	12	17	4	22	24

Weed problems in wheat and rice with yield losses due to different types of weed

From Table 3, maximum yield losses both in wheat (22%) and rice (32%) were found from conventional practices with no straw retention but only 3% yield loss was obtained from 30% SR with PRB in wheat and 6 % in rice, due to severe infestation of broadleaf in wheat and narrowleaf in rice. Malik *et al* (2003) had similar results from residue retention plots with permanent raised bed system.

Effect of weeds on yield and yield attributes

Significant differences were also found in yield and yield contributing characters (Table 4). Lintur 70 WG @ 375 g/ha at two leaf stage produced the highest spike/m², grain/spike, TGW and grain yield which was at par with hand weeding. Lower weed-crop competition resulted in higher absorption of nutrients and interception of sunlight and statistically were the same as hand weeding. Lowest grain yield and yield components were found in control plots i.e. no weeding, due to weed-crop competition from first to last with resulting competition for nutrient, air and sunlight. Hand weeding and Lintur 70WG @ 375 g/ha at two leaf stage produced highest grain yield. Similar results were reported by Verma and Sirvastava (1998). Significantly lowest yield was obtained from the unweeded plot.

Table 4: Yield and yield attributes of wheat variety Shatabdi as affected by different treatments.

Treatments	Rate of application	Plants /m ²	Spike/ m ²	Spikelet/ spike	Grain /spike	TGW (g)
Lintur 70 WG	250 g/ha	240bc	253	18ab	38.6ab	41.1
Lintur 70 WG	300 g/ha	234cde	259	17bc	33.3bc	36.6
Lintur 70 WG	375 g/ha	259a	290	19a	39.5a	42.0
Lintur 70 WG	250 g/ha	234cde	265	16bcd	37.1ab	36.7
Lintur 70 WG	300 g/ha	244b	235	17bc	39.0ab	38.1
Lintur 70 WG	375 g/ha	240bcd	265	17bc	38.7ab	36.7
Lintur 70 WG	250 g/ha	233de	277	18abc	38.5ab	38.2
Lintur 70 WG	300 g/ha	224f	289	16bcd	36.1abc	37.2
Lintur 70 WG	375 g/ha	231e	259	16cd	36.1abc	38.0
2,4 D Amine	1.5 L/ha	216g	283	17bc	36.9ab	38.4
Hand weeding	One operation	264 ab	279	18abc	36.8ab	39.9
Control	No weeding	199h	245	17d	32.6c	34.3
CV(%)		1.69	12.30	4.60	5.45	6.71
LSD(0.05)		6.52	NS	1.36	3.42	NS

Benefits

We calculated returns from reduced weeding cost, with all other input costs kept constant (Table 5). The data reveals that the use of Lintur 70 WG at the rate of 375g/ha sprayed at 25 DAS gives the highest benefit. Benefits over control for the others are: Taka 65750 (8.02 %), hand-weeding Taka 63050 (7.09%), Lintur 70 WG at the rate of 300g/ha Taka 64100 (7.66%) and 2-4, D Amine Taka 61400 (5.49%), per hectare, respectively.

Table 5: Cost related to herbicide uses and returns by some newly developed herbicides

Treatments	Rate of application	Input costs related to weeding				Grain yield (t/ha)	Grain price (Total Taka)	Return (after deducting weeding cost (Tk)
		Herbicide Cost (Tk/ha)	Labor cost for application (Tk/ha)	Rent of sprayer (Tk)	Total (Tk/ ha)			

Lintur70 WG	250 g/ha	1250	250	150	1650	2.70		
							54000	52350
Lintur 70 WG	300 g/ha	1500	250	150	1900	2.80	56000	54100
Lintur 70 WG	375 g/ha	1875	250	150	2275	3.40	68000	65750
Lintur 70 WG	250 g/ha	1250	250	150	1650	3.13	62600	60950
Lintur 70 WG	300 g/ha	1500	250	150	1900	3.30	66000	64100
Lintur 70 WG	375 g/ha	1875	250	150	2250	2.96	59200	56950
Lintur70 WG	250 g/ha	1250	250	150	1650	3.14	62800	61150
Lintur 70 WG	300 g/ha	1500	250	150	1900	2.80	56000	54100
Lintur 70 WG	375 g/ha	1875	250	150	2250	2.86	57200	54950
2,4 D Amine	1.5 L/ha	1800	250	150	2200	3.18	63600	61400
Hand weeding	One operation	-	2,950	-	2950	3.30	66000	63050
. Control	No weeding	-	-	-	-	2.40	48000	48000

RECOMMENDATION

Overall results from the experiment demonstrated that Conservation Agriculture technologies were very essential to control all types of weeds in rice wheat systems. Lintur 70 WG @ 375 g/ha at two leaf stage (10-15 DAS) gave better performance for both weed control and higher grain yield. On the other hand Lintur 70 WG at 375g/ha herbicide was economically better for the control of wheat weeds, giving comparable results with less cost. Therefore permanent tillage system and lintur 70 WG @ 375 g/ha at two leaf stage were recommended for weed control in wheat fields in rice-wheat systems.

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LIQUID CARBON DIOXIDE FOR SELECTIVE WEED CONTROL IN ESTABLISHED TURFGRASS

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ABSTRACT:

Due to public concern amid synthetic pesticides, interest in non-chemical weed control techniques has increased. Typical 'organic' crop production utilizes mechanical weed control including cultivation and hand-weeding, among other practices, which are not practical in established turfgrass systems. Liquid carbon dioxide forms a frost-like layer on plant tissue following application. Depending on the dwell time, the frost-like layer may result in cell membrane destruction and subsequent plant injury. Field and greenhouse experiments were conducted to determine the efficacy of reclaimed carbon dioxide for selective weed control in turfgrass systems. Liquid carbon dioxide was applied utilizing a prototype handheld applicator equipped XR8004 flat fan nozzle calibrated to deliver 16 g carbon dioxide m⁻² s⁻¹, respectively. Treatments included incremental dwell times and were compared to a standard postemergence herbicide. Common annual or perennial turf weeds including annual bluegrass (*Poa annua*), buckhorn plantain (*Plantago lanceolata*), common chickweed (*Stellaria media*), common dandelion (*Taraxacum officinale*), corn speedwell (*Veronica arvensis*), curly dock (*Rumex crispus*), henbit (*Lamium amplexicaule*), large crabgrass (*Digitaria sanguinalis*), and white clover (*Trifolium repens*) were evaluated. Weed control was visually estimated on a 0 – 100% scale. Results indicate greater weed control was obtained with longer exposure times. Further, annual weed species were more susceptible than perennial weeds; however, control varied depending on plant maturity. Finally, prototype modifications including an insulated cone surrounding the applicator revealed a reduction in liquid carbon dioxide necessary for large crabgrass control. These data suggest liquid carbon dioxide may offer a viable alternative to conventional postemergence herbicides for selective weed control in established turfgrass.

KEYWORDS: alternative weed control, turfgrass weed control

INTRODUCTION

Integrated weed management may be defined as the most suitable combination of biological, cultural, and chemical pest control options that yield the most cost-effective, environmentally-friendly, and socially acceptable outcome for a given scenario (Zoschke and Quadranti, 2002). Biological control utilizes living organisms to reduce the population of a species that has risen to the status of a weed (Howart, 1991). Cultural control is the deliberate manipulation of the cropping system or specific production practice(s) to reduce weed populations or avoid injury to crops caused by the presence of weeds (Ashdown, 1977) while chemical control utilizes synthetic xenobiotics for control of undesirable plants.

When comparing the three control categories aforementioned, synthetic pesticides provide the best long-term control in most cases (Busey, 2003). Further, cultural and biological control agents are most impactful when performed over an extended period of time to preventatively keep weed infestations from occurring (Busey, 2003; Rask and

Kristofferson, 2007). In short, chemicals provide land managers with reliable, cost-effective, and efficacious options for weed management. It is for these reasons chemical usage is relied upon heavily by growers in developed countries. Duffy (1998) reported 90% of all corn and soybean grown in the United States were treated with herbicides in the early 1990's. The U.S. Environmental Protection Agency reported in 2007, herbicides accounted for 40% of all synthetic pesticide applied throughout the world. This was over twice the amount of applied insecticides and fungicides (17 and 10%, respectively) (Grobe *et al.*, 2011).

Biological control agents have demonstrated acceptable efficacy on various weed species. Examples include *Xanthomonas campestris* for *Poa annua* L. (Johnson *et al.*, 1996) and *Bipolaris setariae* (Sawada) Shoemaker for *Eleusine indica* control (Figiola *et al.*, 1988). Although these weed management practices have shown some promise, they provide short term control. Further, efficacy is highly dependent on environmental conditions (Busey, 2003).

Compared to traditional row crop weed management, perennial crops including turfgrasses have reduced cultural options for pest management. Practices such as disking tilling, and brushing are destructive processes that cause substantial soil disruption (Hatcher and Melander, 2003). Cultural weed control methods such as flame weeding and ground covers are also not feasible in turfgrass systems due to potential public safety concerns (ex. fire outbreaks) (Fergedal, 1993) and plant-light interception (i.e. covers/mulches) (Bond and Grundy, 2001). Currently, cultural weed management options in established turfgrass systems are limited to hand-removal and holistic turfgrass management. Although hand removal of weeds is safe and efficacious, it is very labor intensive and is cost-prohibitive in most managed turfgrass systems.

Regardless of system, weed management remains an ominous burden for managers without herbicides (Forcella, 2009). This is especially true in systems with reduced cultural control options as biological options are often dependent on cultural management (Busey, 2003; Hatcher and Melander, 2002). Currently, there is a need for better selective, cost-effective and efficacious methods of weed management in settings where synthetic pesticides are not allowed or an environmentally sound option. FrostbiteTM Weed Control System utilizes recaptured liquid carbon dioxide to create frost on targeted plants, instantaneously freezing plant surfaces. As exposure increases, intracellular freezing occurs, leading to membrane destruction and subsequent physiological process disruption (Sakai and Larcher, 1987). The objective of this research was to determine the efficacy of FrostbiteTM Weed Control System for selective weed control in established turfgrass systems.

MATERIALS AND METHODS

Field Research

Research was conducted during 2010 at the Lake Wheeler Turfgrass Research Station in Raleigh, NC, USA to determine the efficacy of liquid carbon dioxide (LCD) for selective weed control in established turfgrass systems. Various weed species, growth stages, prototype designs, and exposure times were evaluated. Experimental units were comprised of established 'Confederate' tall fescue (*Festuca arundinacea* Schreb.) plots

maintained as a home lawn with supplemental irrigation, weekly mowing (10 cm height), and recommended fertilization. Soil type was a Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludults). After treatment, mowing was discontinued for the remainder of the experiment. Treatments were applied with the Frostbite[™] 1 LCD handheld prototype equipped with a single XR 8004 nozzle². Nozzle height was 15 cm and exposed to atmospheric conditions. Application output was 0.036 kg CO₂ sec⁻¹ over a 181 cm² area.

Trial A consisted of a factorial treatment arrangement of four LCD dwell times (6, 15, 30, and 60 seconds) and eight weed species (Annuals: *Lamium amplexicaule* (LAMAM), *Poa annua* (POAAN), *Veronica arvensis* (VERAR), *Stellaria media* (STEME); Perennials: *Trifolium repens* (TRFRE), *Plantago lanceolata* (PLALA), *Taraxacum officinale* (TAROF), and *Rumex crispus* (RUMCR)). Four common annual and perennial weed species were selected to compare annual and perennial species control. Treatments were arranged in a randomized complete block design with three replications and applied March 18, 2010 and a nontreated control was included.

Trial B consisted of a factorial treatment arrangement of four LCD dwell times (2, 5, 15, and 30 seconds) and two growth stages (2-3 tillers and 3-4 tillers) of large crabgrass (*Digitaria sanguinalis* (L.) Scop.). Treatments were arranged in a randomized complete block design with three replications and applied April 16, 2010 and a nontreated was included.

In each trial, control was visually estimated 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 days after treatment (DAT). Control was visually estimated on a 0 to 100% scale (0 = no observed injury and 100 = complete plant death). Data were subject to analysis of variance (ANOVA) and orthogonal contrasts were performed using general linear models (GLM) in SAS to determine the influence of lifecycle. Means were separated according to Fisher's Protected LSD at P = 0.05. Significant treatment by species and treatment by growth stage interactions were detected in Trial A and B, respectively; therefore, simple effect of treatment is presented for each trial. Means were subjected to regression analysis to determine the lethal LCD dwell time to obtain 50% large crabgrass control (LD₅₀) 28 DAT for growth stages to assess improvements from prototype modifications in forthcoming greenhouse research.

Greenhouse Research

Research was conducted in 2010 at the Method Road Greenhouse in Raleigh, NC, USA to determine the influence of growth stage, prototype design, and dwell time for large crabgrass control. Large crabgrass³ was seeded in pots (181 cm² surface area, 2080 cm³ volume) and thinned 14 and 21 days following initial germination to have a final plant count of 5-7 uniform plants. Growing medium consisted of 60% Norfolk clay loam and 40% river bottom sand. Greenhouse day/night temperatures were approximately 31/20 C and supplemental light was provided at 350 µmol/m²/s for 16 hr daily. Pots were irrigated three times a day with overhead irrigation and fertilized weekly with a 20-20-20 soluble fertilizer at a rate of 12.2 kg (N-P-K) ha⁻¹. Treatments were applied with the modified Frostbite[™] prototype equipped with an insulated cone (2,195 cm³) (Figure 1). The modified prototype was also equipped with a single XR 8004 nozzle. Pots were arranged under a structure to create a flush surface surrounding the top of the pots, creating an enclosed treatment area not exposed to atmospheric conditions.

A factorial treatment design including four LCD dwell time treatments (0.5, 1.5, 3.0, and 5.0 seconds) and three large crabgrass growth stages (1-2 leaf, 3-4 leaf, and 1-2 tiller) was used in a randomized complete block design with six replications. Blocks were re-randomized biweekly to negate variation in light quality within the greenhouse. Large crabgrass control was visually estimated 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 days after treatment. Control was visually estimated on a 0 to 100% scale. Data were subjected to ANOVA using GLM and means were separated according to Fisher's Protected LSD at $P = 0.05$. A significant treatment by growth stage interaction was detected; therefore, the simple effect of treatment at each growth stage is presented. Means were subjected to regression analysis to determine an LD_{50} 28 DAT for all growth stages for comparing prototype modifications in previous research.

RESULTS AND DISCUSSION

Field Research

Treatment by species interaction was significant 7 and 28 DAT in Trial A; therefore, the simple effect of treatment for plant species is reported with $\geq 82\%$ annual broadleaf weed control 28 DAT (Table 1). Further, perennial broadleaf weed control ranged from 0 – 68%, 28 DAT. Data at both rating dates suggest lifecycle has a significant ($P < 0.0001$, 7 and 28 DAT) effect on LCD efficacy (Table 2). Similarly, Rifai *et al.* (2002) reported a significant dependence of thermal weeding efficacy on plant lifecycle. Averaged over treatment, control was at least 50% greater for annual weeds compared to perennial weeds at both evaluation dates.

Among annual weed species, it should be noted control was greatest for broadleaf plants (LAMAM, VERAR, and STEME) at all LCD exposure periods 28 DAT. This is consistent with flaming data from Cisneros and Zandstra (2008) who concluded broadleaf weeds were more negatively impacted by treatments than grasses due to greater leaf surface areas and exposed growing points. Leon and Ferreira (2008) reported injury from steam treatments increased as plant leaf width increased and thickness decreased in broadleaf species tested.

The main effect of dwell time is reported 7 DAT for Trial B (Table 3). However, the treatment by growth stage interaction was significant 28 DAT; therefore, the simple effect of treatment at both growth stages is reported. Data from 28 DAT indicate reduced control as plant maturity increases. This trend parallels Forcellas' (2009) results from treating maize weed species with crop debris projected from a sand blaster. LD_{50} values from regression analysis suggest nearly a 25% LCD exposure duration reduction from large crabgrass at 3-4 tillers to 2-3 tillers (Figure 2). Fergedal (1993) observed a similar developmental stage dependence on the response of curlytop knotweed (*Polygonum tomentosum*) exposure to varying amounts of liquid nitrogen and solid carbon dioxide.

Data from this research indicate plant responses to LCD coincide with other thermal and air-propelled weed management practices. As with other weed control practices, perennial weeds are typically more difficult to control. Further, as a given weed species matures it is typically more resilient to control practices. These data indicate exposure to LCD may need to be reduced for feasibility in commercial applications.

Greenhouse Research

Treatment by growth stage interactions were significant 7 and 28 DAT; therefore, the simple effect of treatment for growth stage is reported (Table 4). As with Trial B from the field component of our research, the LD₅₀ for large crabgrass control decreased as plant maturity increased (Figure 3). Although we are not able to directly compare large crabgrass control from greenhouse research with Trial B due to obvious environmental and developmental stage at time of treatment variations, a greater than five-fold reduction in LD₅₀'s using the modified FrostbiteTM prototype suggest the insulated cone surrounding the nozzle body increases LCD efficiency. This is logical due to the rapid volatilization of LCD when exposed to atmospheric conditions.

These results indicate FrostbiteTM may be a viable control option for common annual grass and broadleaf weed species in managed turfgrass and landscape systems. Further, with additional research and refinement, perennial weed species control may be obtained.

SOURCES OF MATERIALS

¹ Frostbite®, Arctic, Inc., 4140 Clemmons Rd, Suite 120, Clemmons, NC 27012.

² Tee Jet®, Spraying Systems Co. Wheaton, IL 60189.

³ Large crabgrass seed, Lorenz's OK Seeds, LLC, PO Box 835, Okeene, OK 73763.

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EARLY DETECTION OF EMERGING INVASIVE ALIEN, *Triplaris americana* IN KWAZULU-NATAL, SOUTH AFRICA

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ABSTRACT

The Ant Tree (*Triplaris americana*) originates from South America, and was first taken to South Africa in the 1970s as an horticultural accent plant. This attractive species has become a popular garden ornamental, but is now considered an emerging Invasive Alien Plant (IAP) in KwaZulu-Natal, South Africa. It produces masses of wind-dispersed seeds that pose a threat to South Africa's rich biodiversity. In South Africa *T. americana* is a declared invader and must be removed from private property.

The Early Detection and Rapid Response programme (EDRR), which is sponsored by the South African Department of Environment has worked in collaboration with the private sector, non-government organisations and governmental institutions to detect and reduce the spread of *T. americana* in KwaZulu-Natal (KZN), resulting in significant progress towards the eradication of this species from KZN.

Ecology of *Triplaris Americana*

Triplaris americana (Polygononaceae) is a popular exotic ornamental tree in KZN, South Africa. Characterized by a smooth, greyish coloured bark, these trees often reach heights of up to 10 meters. This dioecious species produces bright pink flowers on female trees and inconspicuous flowers on male trees between April and September in South Africa. Seeds are enclosed in pinkish-brown 3-winged wind-dispersed fruit. Each fruit encloses a single seed and on germination results in an individual seedling.

T. americana is commonly called the Ant Tree, because of the ants which reside in the bark, flowers and leaves of this species. At least five *Pseudomyrmex* species of ants are found to be associated with the *Triplaris* genus.

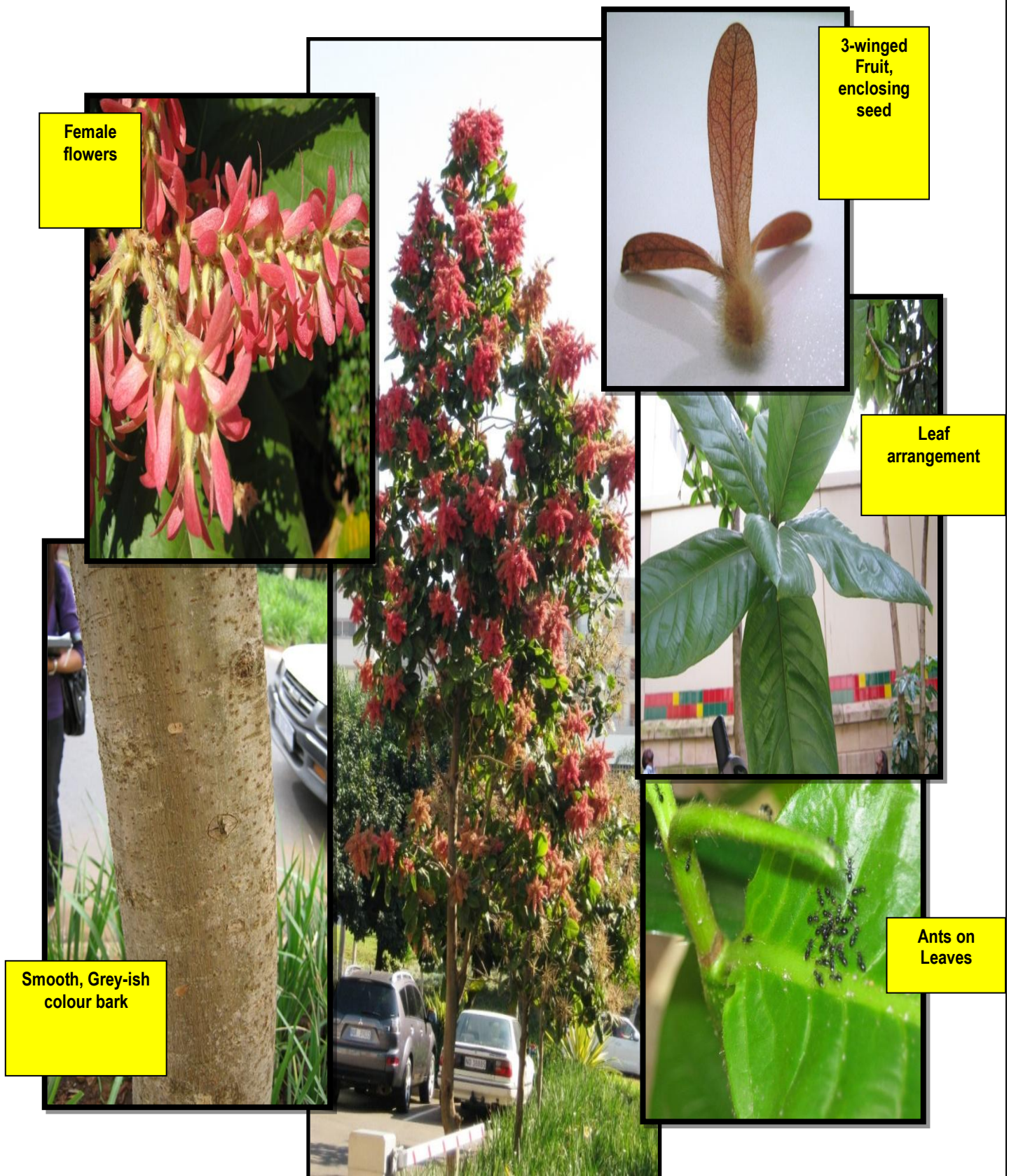


Figure. 1. Adult Female Ant Tree, highlighting different plant features in the sub-set photos

Invasive potential and risk to South African biodiversity

T. americana originates from South America and it is believed that the first Ant Tree introduced to South Africa in the 1970's was for ornamental purposes. The attractive pink female flowers have led to the popularity of Ant Trees in gardening and landscaping in KZN. However, because of the efficient wind-dispersal of the thousands of three-winged fruit, masses of seedlings are produced. It is these offending seedlings that are the major cause for concern as they are capable of jumping the garden fence and spreading along roadsides and water courses, and negatively affecting natural systems.

T. americana is a potential transformer and a declared weed under the South African Conservation of Agricultural Resources Act (CARA; 1983).

EDRR efforts to reduce the spread of this species in South Africa

The EDRR programme, which focuses on emerging IAPs, operates in a number of provinces of South Africa, including the east coast province of KwaZulu-Natal. Concerns about the invasiveness of *T. americana*, were first brought to the attention of the KZN unit of the EDRR programme in May 2009. An initial search on the South African Plant Invader Atlas (SAPIA), revealed only one record of *T. americana* in South Africa. Since then, the EDRR programme has embarked on an intensive search for plants in the area as well as various awareness-raising efforts to educate people about the negative effects of the Ant Tree.

EDRR efforts to reduce the spread of this species in KZN have aimed at altering the mindset of people from positive to negative associations with the tree. Although the escaped seedlings are the real problem for biodiversity, the spread of this species will continue unless all the adults are also removed from gardens and landscapes.

Collaboration with conservation organisations in South Africa has been useful for disseminating information about the Ant Tree, and has led to an increase in the number of reports of new localities. One such example was a collaborative workshop with a non-governmental organization, in which Ant Tree identification training was provided to local municipality horticulture staff, so that seedlings could be removed as soon as they were detected. A second example of collaborative effort was the use of a local municipality website to disseminate electronic information on the Ant Tree to create awareness around this species.

Example of a success story

The Gateway Theatre of Shopping is one of the largest shopping malls in Africa. Seventy four adult *T. americana* trees were detected on the property, growing as part of the

landscape. Relevant personnel at the mall were approached by the EDRR programme in 2009, and since then approximately 30 trees have been removed, and several others have been ring-barked. All *T. americana* trees at the site will ultimately be removed and replaced with an indigenous alternative.

Methods used to engage the public in the management of this species

These have ranged from talks and presentations at workshops and meetings to exhibits at garden shows and the production of pamphlets, posters, newspaper articles and electronic information during 2009 and 2010. Powerpoint presentations have been summarized in (Table 1 below)

Table 1: Powerpoint presentations on *Triplaris americana* made during 2009-2010

Date	Target Audience or Group or Event, for the Presentation
15 July 2009	Working for Water Environmental Training Workshop
18 July 2009	eThekweni Conservancy Association
20 Sept 2009	Custodians for Rare and Endangered Wildflowers (CREW) Summer Rainfall Annual Workshop
8 Oct 2009	Gateway Theatre of Shopping: General Managers and Landscaping Consultants
16 Nov 2009	The Botanical Society of South Africa (KZN Coastal branch)
3 Dec 2009	KZN Invasive Alien Species (IAS) Forum
13 Apr 2010	WfW Project Managers Workshop
13 May 2010	KZN Invasive Alien Species (IAS) Forum
20 May 2010	29 th AGM of the KZN Conservancy Association
1 Sept 2010	Durban University of Technology Horticulture 2 nd year students
8 Sept 2010	Birdlife Port Natal General Meeting

Most of the public engagement targeted audiences with *some* degree of knowledge of botany and invasive alien plants. However, this preaching to the converted approach is insufficient in achieving the ultimate goal of eradication of *T. americana* from KZN.

Due to the popularity of *T. americana* as a garden ornamental, there was a need for a suburb-based case-study to evaluate the response of private-homeowners to the call to eradicate the Ant Tree.

Preliminary results of a study on monitoring of the distribution of *T. Americana* in KZN

The Berea North region in KZN was identified as the study area, and each street was surveyed for Ant Trees in gardens from a car with a driver and one observer. Awareness-raising Ant Tree pamphlets were placed only in postboxes of *private properties* in which

Ant Trees were detected (Ant Trees were also detected in public parks and university grounds, but pamphlets were not administered in these cases, even though these trees and seedlings were recorded). Pamphlets highlighted negative qualities of Ant Trees, provided contact details with instructions to contact the EDRR programme, and also provided details of two indigenous alternatives to *T. americana*. No calls from the public have been received to date, implying that this particular method of public engagement (placing pamphlets in postboxes) was not successful, and corrective steps need to be taken (e.g. paying a visit to the landowner)

Numbers of detected Adult Trees and seedlings per suburb are shown in Figure. 2, and totals for the Berea North region are shown in Figure. 3

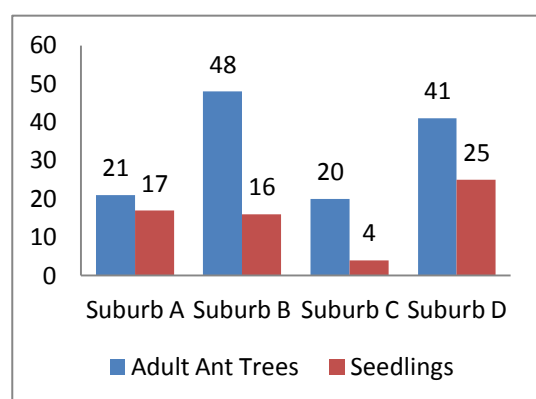


Figure. 2. Numbers of Adult Ant Trees and seedlings detected per suburb in Berea North

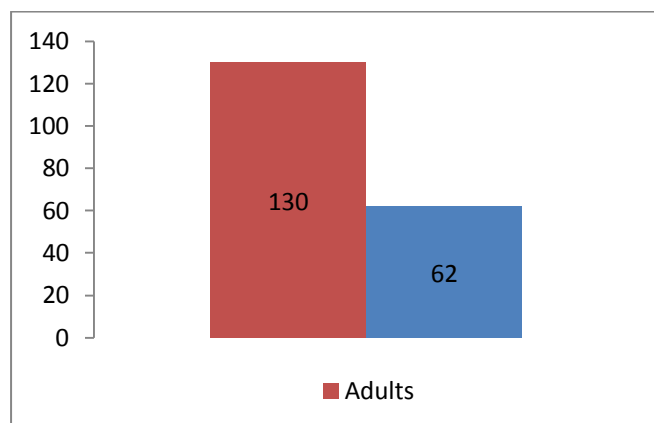


Figure. 3. Total number of Adults and seedlings detected in Berea North region

There were 130 Adult Ant trees and 62 seedlings detected in Berea North, during the drive-by monitoring survey (Fig 3). Most of the Adult Ant trees were detected in Suburb B, whilst most seedlings were found in Suburb D (Fig. 2). This indicates a non-linear relationship between Adult vs Seedlings per suburb, and to re-iterate this point, ratios of seedlings to Adult Trees were calculated in each suburb using the following formula: $\text{Number of Seedlings} / \text{Number of Adults} \times 100$. Results in Fig. 4 below, show that the smallest ratio of Seedlings :Adult trees occurred in Suburb C, i.e. only 1 seedling was sighted for every 5 Adult trees detected.

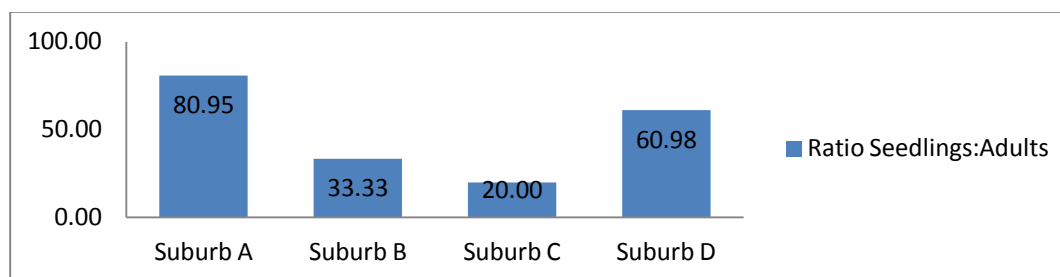


Fig. 4. Ratio of seedlings vs Adults, per suburb

The non-linear relationship between Seedlings: Adults per suburb, and the relatively small number of seedlings detected in total compared to Adult Trees, could be attributed to size: Seedlings are much shorter and in properties surrounded by high fences, would have remained undetected during the survey, but Adult trees which grow up to 10 m high could easily be seen over fences. This is further emphasized by the fact that only 15 out of the 62 seedlings in total, were detected on private property (which tend to be surrounded by high walls or fences).

Due to the different areas of the 4 suburbs, a possible correlation between the number of Adult Ant Trees detected and the area of suburb was investigated and data are presented in Figure. 5 below:

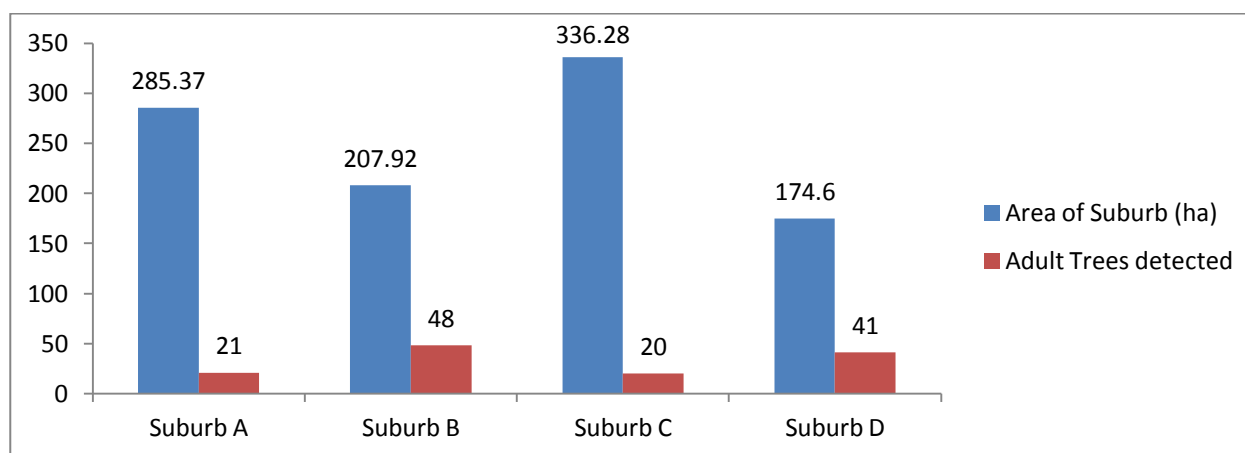


Figure. 5 Suburb area and the number of Adult Ant Trees detected

One would have expected a positive correlation between suburb area and number of Adult Ant Trees, but this was not found to be the case. The largest suburb (Suburb C) actually showed the least number of Adult Ant Trees detected, and quite a large number of trees were detected in the smallest suburb (Suburb D). Since suburbs are displayed in figures according to the order in which they were surveyed (i.e. Suburb A was surveyed first, and Suburb D last), there does not seem to be a decline in research effort over time: i.e. there is no indication of detection withering over time.

PRELIMINARY RESULTS

Although data from this monitoring case study are still being analyzed, preliminary results of an incomplete data set, indicate that:

- a) The response of the general public to the Ant Tree pamphlet was poor, despite the significant number of Ant Trees that were detected in the area. This was to be expected since resistance to change is an inherent trait in most people. Timeous follow-ups are extremely important in public engagement, and time lapses between interlinked methods of public engagement should be minimized to encourage public participation and to strengthen the cause of the study and/or organization. There may also need to be an *incentive* attached to such exercises to encourage public participation.
- b) The implementation of South African Law on IAPs needs to be improved and more information about the relevant Acts need to be communicated to the public. Despite the pamphlets clearly stating that *T. americana* is a declared invader and illegal to have on one's property, this did not prompt the public into action.
- c) Various factors such as: time of day, time of year, number of observers, etc, impact on monitoring surveys and these factors need to be taken into consideration in future such studies: e.g. Simultaneous driving and looking for trees proved to be ineffective and dangerous: There should be at least 2 observers, separate to a designated driver.
- d) Numbers of Seedlings detected during monitoring surveys may not always be truly reflective, as many of them escape detection simply because they are too small to be seen over boundary fences.
- e) Detection is much greater where dense populations of Ant Trees occur. The 2 suburbs with the highest number of Adults detected, comprised localized dense populations on property with no boundary fences, and this would have enhanced detection of the trees.

CONCLUSION

Some species introduced into countries as ornamentals end up as garden escapees, as they flourish and spread into areas where they were not planted. *T. americana* is such a species and its occurrence in KZN needs to be addressed so that it does not spread to other provinces. All *T. americana* trees detected in KZN should be removed, preferably before they set seed. Sites at which *T. americana* have been cleared should be monitored on a regular basis to detect and eliminate any seedlings that may have survived. Due to its popularity as a garden ornamental, buy-in of the public is vital in the eradication of this species from SA. The formation of a *T. americana* working group and collaboration with stakeholders will facilitate eradication of this invasive species.

AN ECOSEMIOTIC APPROACH TO WEED BIOSECURITY

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ABSTRACT

Ecology is a study of the conditions that support life in an *ecos* (Greek for 'home'). Semiotics studies the intellectual frameworks that enable the sciences generally. When we combine these two interests, the result is eco-semiotics, a hybrid methodology that enables us to study the communicational conditions that sustain future life. The paper will engage with the method to focus on the relationships between weed ecology and the human construction of weed meanings. Within this context, it will be argued that the popular call for a 'war on weeds' is largely a human manipulation of affect. The rhetoric is designed to persuade us to malign and efface troublesome plants – to de-mean them. Plants, however, are only troublesome because we have made them so. We create the environmental conditions that generate weeds by imposing our human needs upon their ecosystems. Thus, a 'war on weeds' is really a struggle with our relationship with nature. Viewed in this manner, the varied ways plants respond to our interventions co-facilitate and co-construct our social understandings of ecosystem, either negatively or positively. Given this dialogical repositioning, the paper argues that the ways we re-design ecosystems to meet our future needs should ultimately depend upon our 'eco-literacy'. In a bio-security context, it is not in anyone's long-term interest to promote a 'war on weeds' without also recognising the contingent eco-semiotic frameworks that enable us to appreciate weeds in the first place. If we address the socially constructed aspects of weed preventing and/or controlling, the problem of weeds may eventually be dissolved.

BACKGROUND

Poisoning is easy but nurturing is a craft ...

Isabelle Stengers (2008)

At the scale we have grown used to transforming Australian landscapes to meet our human needs, there are few, if any industries that could claim to have been successful without also causing some degree of environmental dislocation. Environmental dislocations are created through the design and implementation of human displacement activities, such as land clearing, road building, farming, international trade, or any number of other highly valued economic activities. The term 'dislocation' is used here to draw attention to the invisible frameworks and understandings within which the term 'environmental problem' is often embedded. In this paper, we are talking about the dislocation of naturally given plant life into the realm of socially constructed concepts, such as "pollution" and "harm". In this human bio-security domain, whatever life cannot be usefully absorbed into the economy of human design is destroyed, displaced, or simply

overlooked, only to revisit us later. In the case of dislocated plants, the living displacements revisit us as “weeds”.

Weeds are usually considered to be an ‘environmental problem’ because they are ‘plants in the wrong time and place’ (Dwyer 2007). Implicitly, weeds are therefore plants out of sync with humans because our human priorities have made them so. Viewed in this way, weeds can be thought of as plants that have rebelled against the human pressures of industrial land-uses and have become enemies of the value of its operation. Indeed, if this is the case, it might then be said that ‘they’ (the weeds) are responding to the logic of the systems they are enmeshed in and ‘fight back’, or behave like ‘terrorists’ (Barker, 2009). Weeds in this sense are understood to be plants attempting to assert their existence in a coupled ecological / human-affect system. The affect, in the case of weeds, is based in a mobilisation of fear – the fear of plants running amok (Dillon 2007). As such, the popular call for a ‘war on weeds’ is a struggle over the possible future conditions within which plant life can be either befriended (Low, & Fisher 2010), or effaced by human action (Anderson 2010a). As Puig de la Bellacasa (2010) puts it, affectively animated interests, such as ‘fear’, ‘concern’ and ‘care’, have become “intimately entangled in the ongoing remaking of the world” (p. 87).

Securing life

Given that both weeds and the aligned military style of thinking used to address them arise out of human priorities and cultural preferences, weed eradicating and/or preventing are often seen to be highly necessary to securing the future from a human point of view (Trigger *et al.* 2007; Kull & Rangan 2007). Bio-security, in this context, is the securing of a way of life that enables only valued life to survive (Anderson 2010b). Further, the question at issue is also one of how to secure the existence of life that ‘might’ or ‘might not’ be of value at some time in the future (Hinchliff & Bingham 2008).

With respect to weeds and their role in ecosystems, then, a focus on the security of a desired human-economic future must therefore encompass not only what people think or suspect to be dangerous now, but also the realm of the “becoming dangerous” (Dillon 2003 p. 551). From an environmental point of view, however, there are difficulties. Many existing forms of life are not valued by humans (via ‘markets’) and therefore their future must be considered highly uncertain. Securing the future, therefore, also involves securing the human-economic conditions anticipated as necessary to secure future life. Thus, by naming a plant a ‘weed’, humans are extending their present values and assumptions (or lack of) into the future political constitution of life (Anderson 2010a). The central idea underpinning plant bio-security, therefore, is that it is only through the expulsion or control of ‘the dangerous’ or ‘not known’ that order can continue into the future (Watson 1999; Böschén *et al.* 2010).

In the context of a ‘war on weeds’, then, a call to ‘eradicate’ weeds demonstrates a social/political belief that humans have grown powerful enough to actively suppress nature’s dangerous attempts to reject our industrial uses of ecosystem services. Worse, this is often done without assessing the “option value” of the future life these systems might support (Perrings & Brock 2009). Indeed, it could be said that a ‘war on weeds’ is a way of selfishly “freeing one’s own environment of the freedom of the other” (Sloterdijk

2009 p. 49). The human belief at issue, then, is that if humans can annihilate unruly plants by altering and 'securing' the environmental conditions that will eradicate them now, humans can continue with their present industrialised land-use trajectories uninterrupted. Bio-security, in this context, might be said to be a social construct that helps humans make sense of the values they are using (or ignoring), so that they can decide now what is to survive in a future world, or not. 'Ignoring' is included in parenthesis here because the forms of life bio-security 'saves' or attempts to 'eradicate' only become visible when the legitimated constructs used to 'see' or 'ignore' them are challenged. This questioning or 'dissent' (Low 2008) gives rise to powerful negative affective responses, such as anger, revulsion or disgust (Watson 1999).

The term "weed" is, in the above sense, an affectively loaded designation for undesired or 'dis-esteemed' human relationships with plants (Brennan 2008). As Dwyer (2007) has suggested, the term weed may be more a psychological category than a botanical or ecological one. What is perhaps even more prosaic in this context is that people's thinking is part of the process. Human thinking and plant behaviour are both part of the same eco-semiotic reality – a common domain within which the symbolic domain of exchange value is extended into the activities of epistemic cultures usually considered to be 'objective' or 'scientific' (Emmeche 2001, Böschén *et al.* 2010). In this eco-semiotic frame, a "war on weeds" is therefore a war being waged against the very frameworks that enable us to care about, and take responsibility for, plants.

However, as Puig de la Bellacasa (2010) points out, taking responsibility for what and whom we care for does not mean being 'in charge' of them. As shall be argued in more detail shortly, the adequate care of plant life requires a method of finding out the needs of an 'other'. What is being sought in this paper, then, is an understanding of bio-security that goes further than a mere recognition of the complexity of natural ecosystems and the difficulties of valuing (future) life. The paper seeks an understanding that internalises into human actions a 'meta-understanding' (Fisher, 2006).

The meta-approach to be developed here is based in an understanding that we, the understanders, *are* nature. The decisions humans make about life now construct the future possibilities for life. Thus, the degree to which people design environments that nurture future life now may well depend on society's confidence and willingness to support the understandings and methods we use to learn about others' needs. The claim in this paper is that an eco-semiotic method can help create ways of 'being-in-learning'. We make this claim because we believe that it is not in anyone's long-term interest to promote a design for future life stripped of its contingent ecological bases. Indeed, the design of eco-literate interventions is where discussions on what is sustainable (or not) needs to focus.

As Latour has argued, "... to design is always to *redesign* ... there is always something *remedial* in design" (2009, p.5, original emphasis).

A war 'economy' for weeds?

As we have seen so far, the 'war on weeds' analogy serves a number of human social functions. As discussed above, its use necessarily summons questions about how we go about deciding upon the future political constitution of (plant) life (Latour 2004). In the present eco-semiotic context, we suggest another purpose entailed in the use of the war

analogy. Its use serves to shift human expenditure in certain preferred directions. As is the case in any 'real' war situation, "War!" effectively shreds our normal investment priorities – affect is mobilised to create the urgency needed to bulldoze away the messy contingencies that support future life. One of the most overlooked consequences of this manipulation of affect therefore, is that it disconnects the trajectories and social priorities that give rise to weeds from the costs (social and ecological) of controlling or preventing them. As such, the taxation imposed by government to prevent and/or control weeds is no longer transparently connected to the dislocating social activities that give rise to weeds. The disconnecting social activities are therefore not subject to social critique. Put in economic terms, we might say that there is a 'persistent market failure'. The analysis undertaken here, however, suggests that what is really persistent is a lack of ecological literacy.

The centrality of 'the war' analogy in weed discourse therefore largely explains why weed preventing and/or controlling presently attracts mass market support and commands the allocation of significant social resources. For example, the wholesale value of herbicide sales in Australia for 2008-09 – a drought year – was \$1.1 billion (APVMA 2010). As this figure demonstrates, not only do humans invest a great deal of their time and money extracting victories 'over' nature, but they are also willing to spend a great deal of time and money 'protecting' their preferences for a limited range of life – after all, the purpose of herbicides is to efface future life that 'threatens' prevailing human priorities.

What perhaps needs to be understood more clearly, therefore, is how partial the understanding underpinning the 'war on weeds' analogy really is. Circumspection is required.

The human controlling of weeds is usually considered to be a major human achievement. Indeed, many techno-optimists believe our 'conquest of nature' has *almost* been secured. As it is now becoming clearer, the 'almost' is an area of activity that the 'war on weeds' analogy would have us repress. When we step out of this psychological repression, the purported successes generated by the chemical 'weapons' used to kill weeds can be more clearly evaluated. We then see that the successes are limited as they are increasingly being threatened by weedy 'resistance'. Weeds are adapting to our intrusions via co-adaptive (or 'selective') 'fleeings' (Deleuze & Guattari 1987 [1980]). As at May 2010, there were 195 weed species *known* to have evolved herbicide resistance (Heap 2010).

As a consequence of this 'fight back', expensive 'counter-terrorist' practices are now required to maintain the continued use of herbicide weapons, such as glyphosate, the world's most widely used herbicide (Duke & Powles, 2008; Anderson 2010c). This is becoming more the case as our 'fight' against weeds (and the weed's 'fight-back') begins to incorporate understandings of the latest 'genetically engineered weapons' (Dillon 2003; Anderson 2008; Gressel 2010). These developments will make the task of understanding weeds more complex and potentially more dangerous. The world of the plant (and perhaps what it has to offer) needs to be considered if we are to live with plants more harmoniously. As Anderson (2010c) notes, counterinsurgency doctrine is based on a "different logic of enmity" (p. 213). The distinctions between a 'good' and a 'bad' plant no longer hold: the new counterinsurgency emphasis is entirely anticipatory. It focuses on whether a friendly plant could become an enemy in the future, or *vice versa*.

While counterinsurgency thinking offers hope of a more dialogical approach, the difficulty is that the political and policy infrastructures put in place to support the privileging of some plants and the effacing of others are made difficult to change (Fisher 2006). Biological life has been deliberately and systematically tangled with a powerful 'way of life' (Anderson 2010c). As noted earlier, this situation is made even more difficult because human bio-security is increasingly being pursued via a belief that life can be assigned contractually to the future. This is achieved through the rhetorical arts that persuade us as to which contingent dangers we should value as 'growth opportunities', and which are to be ignored or downplayed, or more literally, 'eradicated' (Pottage 1998; Clift 2009; Böschen *et al.* 2010).

The above assumptions about the future constitution of life need critical evaluation. For example, the deployment of more sophisticated molecular weapons and engineered life forms to combat weeds (life forms which are also 'risky') further transforms the so-called 'growth' economy into something that is highly *uneconomic* (Daly 2008). Indeed, under the influence of this combative style of thinking, the task of securing bio-security will continue to demand vast *defensive* expenditures. This will compound the difficulties involved in determining which life forms sustain *both* our ways of living and our ecosystems. We will increasingly be asked to decide which forms of life must be effaced because they refuse our remedial re-designs, and which can be economically conserved within the scope of our industrial interventions.

CONCLUSION

We have argued that the human factors driving bio-security should properly be derived from two main sources. First, plants are responding to our industrial interventions in ways that are becoming increasingly inconvenient (unpredictable) and costly to human decision-makers. In this first sense, then, the source of the concern is real – ecosystems are responding to human interventions in ways that humans did not (or did not want to) anticipate.

The second source is related to the first but is generated by humans alone. In the second sense, social institutions use the dislocations caused by industrialised activities as signals that can be manipulated to cultivate an anxiety amongst the economy's constituents. This anxiety is encouraged and facilitated (for example, via slogans such as, "declare war on weeds") so that the economy can command the necessary resources it needs to continue to grow and to find new markets.

It was argued, however, that economics is largely a rhetorical art controlled by an elite self-interest (Clift 2009; Allan 2007). In this latter sense, then, the issue of weed invasion(s) and/or weed preventing is framed as an economic *opportunity*, that is, if the expense of the dislocation can be pushed to general taxation rather than linked directly to the dislocating activity.

The mistaken understanding that maintains the above situation is perhaps most centrally a belief that biological values are intrinsically different from economic values (e.g., Sinden & Griffith 2006). In this split understanding, decision-makers can solve supposed 'environmental dilemmas' by simply ignoring biological factors, or by privileging elite social priorities. Under the influence of the former, ecosystems are destroyed or impoverished, and under the influence of the latter, herbicide sales become a sign of economic growth rather than an indicator of worsening ecological dislocations.

Human decision-making, in the case of weeds, should logically involve both an assessment of biological responses and a concern with human satisfactions, that is, if the decisions made are to be defensible in any pragmatic sense. Put another way, biological interactions generated through habitat dislocations generate the nuisance plants we call weeds, but it is humans that decide the social contingencies that are important to human welfare. In fighting a war against weeds, we are at war with ourselves.

In summary, in the above eco-semiotic analysis we were able to identify a need to move the focus to learning and transformation rather than vilification and the manipulation of affect. We saw that the frameworks we use to understand and construct our relationships with plants need to be reviewed and revised in order to gain insight into how we create our own difficulties with plants. Doing so, it was argued, is preferable to remaining fixated on winning a final 'victory' over unwanted plant life, especially if that victory would only serve the short-term horizon of an industrial self-interest. Thus, we need to assess whether the resources allocated to addressing the social issues that arise out of human-plant 'cohabitation' can be pragmatically justified on *both* social and biological grounds (Latour 2005 [2004]). Our conclusion is that an essential part of any bio-security strategy for human-plant cohabitation ought to be an investigation into how to 'befriend weeds' (Low & Fisher 2010). After all, if we are unable to live with the characteristics of plants we call 'weedy', we will also be unable to live in harmony with eco-system. The practice of denying and fighting against the human legitimacy of non-human worlds is an un-caring and unsustainable way forward.

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INTEGRATED CONTROL OF TROPICAL SPIDERWORT (*Commelina benghalensis* L.) IN TANGERINE ORCHARD

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ABSTRACT

Tropical spiderwort (*Commelina benghalensis* L.) is one of the most troublesome weed species in tangerine orchards of Thailand. Due to the continuous emergence pattern of tropical spiderwort, integrated control will be required. Two field studies were conducted in Chaing Mai province to evaluate the effectiveness of post- and pre-emergence herbicides on tropical spiderwort. Firstly, glufosinate-ammonium at 0.563 kg a.i./ha was the most effective herbicide giving complete control of *C. benghalensis* at 4 WAT. In contrast, glyphosate at 1.8 kg ai/ha was poorly effective on *C. benghalensis* with less than 34% control at 2 WAT and no control at 4 WAT. Paraquat at 0.856 kg a.i./ha gave an excellent control for two weeks but the weed could regenerate new shoots from the remaining stems at 4 WAT. Secondly, two pre-emergence herbicides, diuron and indaziflam at the rates of 1.5 and 0.075 kg ai/ha were applied after weed removal. The results showed that indaziflam was very effective on *C. benghalensis* with only 6 ± 2.6 seedlings/m² at 4 WAT. whereas diuron gave a moderate control of *C. benghalensis* resulting in 49 ± 23 seedlings/m². In conclusion, hoe weeding prior to removal of the vegetative stems from the field and followed with application of glufosinate ammonium tank mixed with indaziflam gave an effective control of *C. benghalensis* and other weed species without injury to tangerine plants. However, herbicides with different mode of action may be required to prevent herbicide resistance.

Keywords: Tropical spiderwort, *Commelina benghalensis* L., integrated control, tangerine

INTRODUCTION

Tropical spiderwort (*Commelina benghalensis* L.) was reported as a noxious weed in 28 different countries and 25 different crops such as maize, soybean, cotton, banana, sugarcane, tropical grasses and legumes (Holm *et. al.* 1977). It can behave as a perennial in the tropical and sub-tropical lowlands but only as an annual in more temperate zones. The weed occurs from sea level to 2500 m (Wilson, 1981).

It has been estimated that one plant of *C. benghalensis* could produce as many as 1,600 seeds (Wilson 1981). It roots readily at the nodes of the creeping stems especially if cut or broken. Its ability to grow rapidly from the vegetative cuttings makes this weed difficult to

control. When the same herbicide and cultural program is maintained over time its population may build up until it infests the entire grove to become the dominant weed.

Tangerine is mainly grown in the northern part of Thailand where climate is cooler. In 2009, the plantation area was 36,705 ha with annual production of 80,110 ton/ha (Anonymous 2010). In the last ten years, glyphosate provides effective control of most weeds in the orchards except *C. benghalensis*. Consequently, it thrives and becomes dominant with a larger seed bank. To date, *C. benghalensis* has become a dominant weed in the tangerine orchards so that gardeners have to pay higher operation costs.

Paraquat is often used to control *C. benghalensis* but the weed can rapidly recover from the stems during the wet season. Repeated use of the same herbicide may lead to the development of herbicide resistance. This study aimed to develop integrated weed management in citrus orchards in order to keep *C. benghalensis* under control.

MATERIALS AND METHODS

Three field experiments were conducted in Fang District, Chiang Mai province during 2009 and 2010. An eight-year old tangerine plantation heavily infested with *C. benghalensis* was used.

Post-emergence herbicides

Three post-emergence herbicides treatment included no herbicide, and glufosinate-ammonium, glyphosate and paraquat at 0.563, 1.8 and 0.856 kg ai/ha, respectively. Treatments were arranged in a randomized complete block design with six replications. Individual plot size was 12 x 8 m. Herbicides were applied by knapsack sprayer equipped with flat fan type nozzle giving a spray volume of 375 L/ha.

At 15 and 30 days after application (DAA), visual estimate of weed control and crop injury were made on a scale of 0 to 100%, where 0 represented no control or no injury and 100 represented complete control or plant death. Green tissue above ground was cut in an area of 1x1 m², the fresh weight recorded, and the plant tissue then oven-dried at 70°C for 48 hours and the dry weight measured. Means were separated with Least Significance Difference Test (LSD at $p \leq 0.05$) when significant differences among treatments were detected.

Pre-emergence herbicides

To control seed germination of *C. benghalensis*, two pre-emergence herbicides treatment were tested: no herbicide; diuron and indaziflam at 1.5 and 0.075 kg ai/ha, respectively. Treatments were arranged in a randomized complete block design with six replications. Individual plot size was 12 x 8 m². Herbicides were applied by knapsack sprayer equipped with flat fan type nozzle giving in a spray volume of 500 L/ha. At 30 days after application (DAA), density of *C. benghalensis* seedlings were counted in two sampling plots of 0.5x0.5 m² in each replicate.

RESULTS

Post-emergence herbicides

Glufosinate-ammonium gave an excellent control of *C. benghalensis* but glyphosate was poorly effective at 2 WAT after application. Paraquat was less effective than glufosinate-ammonium, giving a high fresh and dry weight of *C. benghalensis* at 30 days after herbicide application (Table 1.).

Pre-emergence herbicides

At 30 days after pre-emergence herbicide application, grass weeds (*Digitaria sanguinalis* (L.) Scop., *Eleusine indica* (L.) Gaertn., *Echinochloa colona* (L.) and *Leptochloa chinensis* (L.) Nees) and broadleaved weeds (*Amaranthus viridis* (L.), *Physalis minima* L. and *Ageratum conyzoides* L.) were found in the untreated control in a total density of 2,517 plants/m² (Table 3.). In untreated plots, density of *C. benghalensis* was very high, ranging from 103 to 155 plants/m² which is enough to cover the ground within four weeks after hoe weeding. When indaziflam at 0,075 kg ai/ha was applied, no other weeds were present and only 6 ± 2.6 plants/m² of *C. benghalensis* were observed. In addition, diuron at 1.5 kg ai/ha gave satisfactory control of general weeds but provided only moderate control of *C. benghalensis* with 49 ± 23 plants/m² (Table 3.). It may be practical to introduce an application of glufosinate-ammonium at four weeks after germination to eradicate all weeds at an early stage.

DISCUSSION

Hoe weeding is not an appropriate method for *C. benghalensis* as it results in cut vegetative parts and these cuttings can regenerate within two weeks in wet seasons and under sprinkler irrigation. Also, hoeing often destroys the feeder roots of citrus trees that are responsible for absorbing nutrients, water, and oxygen from the top soil. Regardless of the cost of labor, herbicide use provides benefits from both the economic and the grove floor management aspect. Equipment and operational costs are generally reduced compared to those for mechanical, tillage, or mowing operations. Proper herbicide applications can minimize injury to citrus tree trunks and roots compared to mechanical hoeing. Injured tree trunks and roots are more susceptible to attack and injury by soil-borne pest and diseases

When the same herbicide and cultural program is maintained, over time the weed population may build up until it infests the entire grove and becomes the dominant weed species. However, repeat use of the same mode of action or management practices may lead to changes in abundance and composition of weed floras in the orchard. Culpepper (2006) studied weed shifts in glyphosate-resistant corn, cotton and soybean and found that some broadleaved weeds and sedges became more problematic after a few years of glyphosate application. Those weeds were *Amaranthus tuberculatus*, *A. rudis*, *C. benghalensis*, *Ipomoea hederacea*, and *Cyperus* species.

Removal of *C. benghalensis* from the tangerine orchard will indirectly protect tangerine trees from some pests. It was reported that *C. benghalensis* is an alternate host of several soil-borne plant pathogens and nematodes (Davis *et al.* 2006). In this study, a high

number of american bollworm (*Helicoverpa armigera*) populations were observed where *C. benghalensis* had become dense in the orchard. Caterpillars caused obvious damage to young flowers and fruits of tangerine. When insecticide is applied to control american bollworm, they will drop off the trees and survive on *C. benghalensis*. Later they will climb up the trees again and damage the young fruits.

In conclusion, an integrated management using herbicides with different modes of action, hoe weeding and mowing would be appropriate to control tropical spiderwort.

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Table 1. Weed control (%), fresh and dry weight of *C. benghalensis* in tangerine orchard at two weeks after treatment (WAT).

Treatment	Rate (kg ai/ha)	2 WAT		
		Weed control (%)	Fresh weight (g/m ²)	Dry weight (g/m ²)
1. Glufosinate-NH ₄	0.563	83	1,041 a ^{1/}	134 a
2. glyphosate	1.80	34	2437 b	254 b
3. Paraquat	0.856	55	1,109 a	129 a
4. Untreated control	-	0	4,160 c	356 c
<i>F test</i>		-	**	**
LSD _{0.05}		-	653.1	54.1
C.V. (%)		-	19.39	16.09

^{1/} Mean in the same column followed with the same letter are not significantly different according to LSD test (p = 0.05).

Table 2. Weed control (%), fresh and dry weight of *C. benghalensis* in tangerine orchard at four weeks after treatment (WAT).

Treatment	Rate (kg ai/ha)	2 WAT		
		Weed control (%)	Fresh weight (g/m ²)	Dry weight (g/m ²)
5. Glufosinate-NH ₄	0.563	100	0 a	0 a
6. glyphosate	1.80	0	4,162 b	536 b
7. Paraquat	0.856	0	2,746 b	1,016 bc
8. Untreated control	-	0	4,436 c	1,514 c
<i>F test</i>		-	**	**
LSD _{0.05}		-	4,356.3	487.5
C.V. (%)		-	26.64	13.46

^{1/} Mean in the same column followed with the same letter are not significantly different according to LSD test (p = 0.05).

Table 3. Effect of pre-emergence herbicides on number of weed seedlings emerged under plant canopy at four weeks after herbicide treatment (WAT).

Weed species	Number of weed seedlings at 4 WAT		
	Untreated control ^{1/}	Diuron	Indaziflam
<i>C. benghalensis</i>	129 ± 26	49 ± 23 ^{2/}	6 ± 2.6
Grass weeds	104 ± 15	0 ± 0	0 ± 0
Broadleaved weeds	2,517 ± 191	42 ± 23	0 ± 0

^{1/} Untreated control was farmer practice to control weeds by hoe weeding and no removal of vegetative cuttings.

^{2/} data were the average ± standard error of six replicates

DETERMINATION OF WEEDY RICE (*Oryza sativa* f. *spontanea*) CONTAMINATION IN RICE SEED LOTS

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ABSTRACT

Weedy rice (*Oryza sativa* f. *spontanea*) has become a noxious weed in direct seeded rice and is widespread throughout the central plain of Thailand. It spread from infested areas to clean areas primarily as contaminants in rice seed lots. To determine contamination of weedy rice in seed, ninety three samples of five rice varieties were collected from farmers and local commercial seed producers. Three morphological characters, i.e. straw with brown strip hull, red pericarp and seed awning, were used to determine the extent of weedy rice contamination. It was indicated that only 18 samples (19.4%) of total 93 seed lots were detected as contaminated seed lots when characterized by seed morphology. Next, 35 samples with seed morphology of crop rice variety were selected and sown in pot experiment, ten plants per sample. DNA analysis was performed using four microsatellite markers. It was found that 14.3% of the samples were contaminated with wild rice alleles. Therefore, seed morphology alone could not efficiently detect weedy rice contamination in rice seed because of its mimicry of crop rice.

Keywords: weedy rice, *Oryza sativa* f. *spontanea*, contamination, DNA analysis.

INTRODUCTION

In 2001, weedy rice (*Oryza sativa* f. *spontanea*) was found to be a noxious weed in direct seeded rice with an area of infestation of less than 100 ha (Maneechote *et al.* 2004). It took only a few years to spread throughout the central plain of Thailand with an infestation area of 10,000 ha. To date, it can be found in most direct wet- and dry-seeded rice growing system over an area greater than 500,000 ha (Maneechote 2009).

It spreads from an infested area to a clean area primarily as contaminants either in rice seed lots or on combine harvesting machines. As farmers grow rice two to three seasons per year, very few farmers can keep their own seeds for the next crop. Once they have to purchase from the local seed producers, the risk of obtaining contaminated seeds increases. In the past five years, farmers could easily distinguish the contamination of weedy rice in rice seed lots because weedy rice has very different characters such as long awns, black or brown seed coat and red pericarp.

Niruntrayakul (2008) reported that weedy rice in Thailand is a hybrid between crop rice (*O. sativa* L.) and common wild rice (*O. rufipogon* Griff.) resulting from gene flow. As gene

flow is an ongoing process, weedy rice can morphologically mimic crop rice through straw seed coat and white pericarb. Consequently, these weedy rice seeds have been sown and caused reduced rice yield in production. To help farmers, a method to determine weedy rice contamination in rice seed lots has been developed using seed morphology and DNA analysis.

MATERIALS AND METHODS

Seed samples

Ninety-three seed samples of five commercial rice varieties, Suphanburi 1 (SP1), Chainat 1 (CNT1), Chainat 80 (CNT80), Pisanuloke2 (PL2) and Pathumtani1 (PTT1) (Table 1.) were collected from farmers and local commercial seed producers. One hundred seeds from each sample were visually inspected for contamination by the presence of awn, brown strip hull and red pericarb. Then 35 seed samples that had appropriate seed morphology of for the rice variety were selected and sown in pots, 10 plants per sample. At tillering, leaf samples from each plant were collected for DNA analysis.

DNA analysis

DNA was extracted from dry leaf samples using modified method from Doyle and Doyle (1987) and the PCR reactions performed following the description of Panaud *et al.* (1996). DNA of pure seed of each crop rice variety and common wild rice population were included. Microsatellite markers with four SSR primer pairs; RM1, RM341, RM444 and RM586 were used.

Primers	Chromosome no.	Repeat Motif	Base sequences	Anneal Temperature
RM1	1	AG)26	F:GCGAAAACACAATGCAAAAA R:GCGTTGGTTGGACCTGAC	55 °C
			F:CAAGAAACCTCAATCCGAGC	55 °C
RM341	2	(CCT)20	R:CTCCTCCCGATCCCAATC	
			F:GCTCCACCTGCTTAAGCATC	55 °C
RM444	9	(AT)12	R:TGAAGACCTTGTTCTGCAGG	
			F:ACCTCGCGTTATTAGGTACCC	55 °C
RM586	6	(CT)23	R:GAGATACGCCAACGAGATACC	

F: Forward Primer R: Reverse Primer

Amplification of DNA was performed in 20 µl reaction consisted of 20-50 ng DNA, 0.25 mM of each dNTP, 2% formamide, 0.2 µM of each primers and 0.5 unit of Taq DNA polymerase in reaction buffer [10 mM of Tris-HCl pH 8.5, 50 mM KCl, 1.5 mM MgCl₂, 0.1mM EDTA, 50%(v/v) glycerol]. The amplified polymorphism alleles were distinguishable with the electrophoresis in 10% Polyacrylamide Gel Electrophoresis (PAGE).

RESULTS AND DISCUSSION

About 24.7% of 93 samples had some levels of seed contamination (Table 1). The characters found were consistent with weedy rice seeds reported in this area, i.e. straw with brown strip hull, red pericarp and seed awning (Maneechote *et al.* 2004). SPR1 variety showed the highest level of seed contamination because it was very popular among farmers due to moderate resistance to brown planthopper. No visual contamination was found in both CNT 80 and PTT 1 seed samples.

For DNA analysis, alleles from common wild rice were detected in progenies within seed samples morphologically indistinguishable from the crop variety (Table 2.). The rate of contamination ranged from 7.4% in SPR1 to 50% of PSL 2 and none in CNT 80. This clearly indicated that weedy rice in Thailand results from gene flow between crop and common wild rice.

Detecting weedy rice seed contamination by seed morphology was a rapid, easy and inexpensive method. However, seed morphology alone could not efficiently detect weedy rice contamination in rice seed when they mimic the crop rice. DNA analysis with alleles that can distinguish crop rice from wild rice could increase the accuracy of detection of weedy rice contamination. Further study is needed to verify more specific primers to reduce the cost and time of determination.

ACKNOWLEDGEMENT

We would like to thank the McKnight Foundation for financial support.

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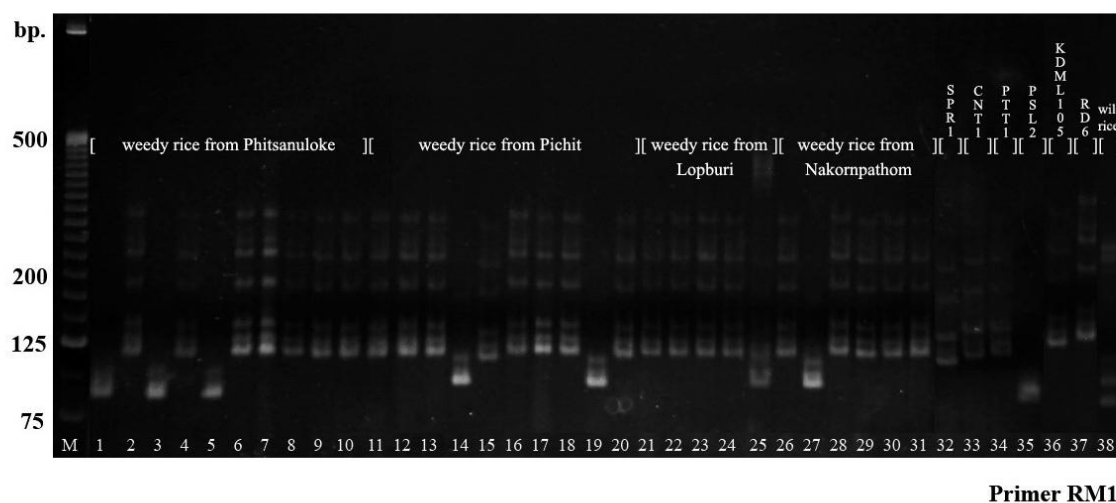
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Table 1. Number of samples with contaminated seeds when determined by seed morphology, 93 samples (100 seed/sample).

Rice Variety	Total	Seed Characteristics			Sample Contaminated with at least one trait	%
		Brown strip hull	Red pericarb	Awned		
SPR 1	59	2	3	16	19	32.2
CNT 1	10			3	3	30
CNT 80	8				0	0
PSL 2	10		1	1	1	10
PTT 1	6				0	0
Total	93	2	4	20	23	24.7

Table 2. Number of samples with contaminated wild rice alleles as determined by four microsatellite markers, 35 samples (10 plants/sample).

Rice Variety	Total	RM1	RM341	RM444	RM586	Sample with contaminated with at least one allele	%
SPR 1	27			2		2	7.4
PSL 2	6	1	3	3	3	3	50
CNT 80	2					0	0
Total	35	1	3	5	3	5	14.3



Picture 1. Microsatellite amplification products from four weedy rice and six crop rice and common wild rice genomic DNA with primer RM1. *Lane 1-10* weedy rice from Phitsanuloke province, *Lane 11-20* weedy rice from Pichit province, *Lane 21-26* weedy rice from Lopburi province, *Lane 27-32* weedy rice from Pichit province, *Lane 33* Supanburi 1, *Lane 34* Chainat 1, *Lane 35* Pathumthani 1, *Lane 36* Khao Dok mali 105, *Lane 37* RD 6, *Lane 38* common wild rice.

PREDICTION OF RICE YIELD LOSS FROM RICE - *A. indica* (*Aeschynomene indica* L.) COMPETITION IN TRANSPLANTED RICE CULTIVATION

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ABSTRACT

Field experiments were conducted to predict rice yield losses caused by *Echinochloa crus-galli* (L.) P.Beauv., *Bidens frondosa* L. and *Aeschynomene indica* L. at a range of plant densities under machine transplanted rice cultivation in different regions of Korea in 2006, and to determine their economic threshold levels (tE).

All data were fitted to Cousens' rectangular hyperbola to estimate parameters for predicting rice yield loss. The rice yield loss models of *Bidens frondosa* L. was predicted as $y = 5.43/(1 + 0.0113x)$, $R^2 = 0.963$ and *A. indica* was $y = 5.47/(1 + 0.0332x)$, $R^2 = 0.976$. The mean competitiveness represented by the parameter, whose reciprocal ($1/\beta$) is a weed density reducing crop yield by 50%, of *E. crus-galli*, *B. frondosa* and *A. indica* were 0.01552, 0.01113 and 0.0332 in normal-season machine transplanting of Korea, respectively and Single year mean economic thresholds (Ets) of *E. crus-galli*, *B. frondosa* and *A. indica* calculated using Cousens' equation were 1.5, 2.5, and 0.7 plants m⁻², respectively.

Key words: economic threshold; interference, weed density

INTRODUCTION

Since uncontrolled weeds can lead to rice yield losses as high as 80% (Smith 1988), weed control is an essential and intensive component of rice production. Accurate prediction of weed-crop interactions is required for integrated weed management (Swanton and Murphy 1996). Mathematical models that summarize the quantitative knowledge of the impact of weed competition on crop yield can provide useful information to support weed management decisions (Vandevender *et al.* 1997). Much effort has been made to develop such mathematical weed-crop interference models (Cousens 1985; Kropff and Spitters 1991), which are commonly used to quantify competitive relationships and predict yield loss. Among those models, the rectangular hyperbola based on weed density (Cousens 1985) has been most widely used to predict crop yield losses as a function of weed density in various crops, such as wheat (Kim *et al.* 2002), soybean (Cowan *et al.* 1998) and maize

(Lindquist *et al.* 1996). Little effort has been made to investigate relationships of rice-weed competition, although Lindquist and Kropff (1996) introduced an ecophysiological model for irrigated rice-*Echinochloa* competition. Recently Ni *et al.* (2004) analyzed competition between wet-seeded rice and *E. crus-galli* using a response-surface model based on the rectangular hyperbola.

This study was also conducted to investigate the competition relationships of *E. crus-galli*, *B. frondosa*, and *A. indica* with transplanted rice using the rectangular hyperbola as a prediction model, and to determine the economic threshold levels for *E. crus-galli*, *B. frondosa*, and *A. indica* in a transplanted rice cropping system.

MATERIALS AND METHODS

Field experiments

Field experiments were conducted to evaluate competition effects of *E. crus-galli*, *A. indica* and *B. frondosa* on transplanted rice in major rice cropping regions in Korea. Experiments with *E. crus-galli*, *A. indica* and *B. frondosa* were conducted in Daegu, and Suwon in 2006. The experiments consisted of three replicates of a completely randomized block design. The plot size was 2 m × 2 m. Thirty-day-old-seedlings of rice (*Oryza sativa* cv. Ilmibyeo) were transplanted at a density of 23.8 hills m⁻² (14 × 30 cm² space) equivalent to about 72 rice seedlings m⁻² on 25 May in 2004. The densities of *E. crus-galli* and *A. indica* were 0, 1, 8, 24, 48, and 96 plants m⁻². The densities of *B. frondosa* were 0, 1, 8, 48, 96, and 192 plants m⁻². The plant densities were artificially adjusted by sowing seeds of *E. crus-galli*, *A. indica* and *B. frondosa*, and transplanting, or hand-weeding to remove naturally occurred background weeds.

Fertilizer was applied as a basal release with N, P₂O₅, and K₂O of 55, 67, and 225 kg ha⁻¹ respectively before harrowing, followed by top-dressing of 22 kg ha⁻¹ of N at the tillering stage of the rice, 10 days after transplanting (DAT), and 33 and 29 kg ha⁻¹ of N and K₂O at the panicle initiation stage of the rice. To investigate competition between rice and the weeds, the number of rice tillers was recorded every 20 days until 100 DAT, and dry weights of the rice and the weeds were measured after sampling from an area of 0.5 m² at 30, 60 and 90 DAT in Suwon in 2004. At maturity, rice was sampled from an area of 1.0 m² and grain yield measured after polishing.

Prediction model and statistical analyses

Rice grain yields were fitted to the following equation (Figure 1.) rectangular hyperbola (Cousens 1985), to estimate parameters for predicting rice biomass and yields as a function of weed density.

$$Y = \frac{Y_o}{1 + \beta X}$$

Figure 1. Rectangular hyperbola.

Where Y_0 is weed-free rice yield (t ha^{-1}), β is a measure of weed competitiveness (a weed density of $1/\beta$ reduces the rice yield by 50%) and X is weed density.

Economic thresholds (ET) of *E. crus-galli* and *A. indica* and *B. frondosa* were estimated by equating the cost of controlling these weeds with the value of rice yield gained by herbicide application. Their calculation was based on the equation developed by Cousens (1987) (Figure 2.):

$$ET = C_h + C_a \left[\frac{C_o PLH}{Y_0} \right]$$

Figure 2. Cousens equation.

Where C_h is herbicide cost ($\text{US\$ ha}^{-1}$), C_a is application cost ($\text{US\$ ha}^{-1}$), Y_0 is weed free rice yield (t ha^{-1}), P is value per unit of crop ($\text{US\$ t}^{-1}$), L is proportional loss per unit weed density, and H is herbicide efficacy, a proportional reduction in weed density or weed biomass by the herbicide treatment.

All statistical analyses were conducted using Genstat (Genstat Committee, 2002).

RESULTS AND DISCUSSION

Prediction of rice yields

By fitting the rectangular hyperbola (Figure 1.) to rice yield, weed-free rice yield (Y_0) and weed competitiveness (β) of the three weeds were estimated. For rice in competition with *E. crus-galli*, the estimated weed-free yield was 5.43 t ha^{-1} (Figure 3.) and for competition with *A. indica* and *B. frondosa* was 5.47 and 5.43 t ha^{-1} , respectively. The competitiveness (β), whose reciprocal ($1/\beta$) is the weed density that reduces crop yield by 50%, of *E. crus-galli*, *B. frondosa* and *A. indica* was 0.0155 , 0.01113 and 0.0332 respectively for normal-season machine transplanting in Korea. Based on parameter estimates and equation 1, rice yield as a function of *E. crus-galli* competition was calculated (Figure 3). There is good agreement between calculated and observed yield as *E. crus-galli* density increases, showing that competition between rice and *E. crus-galli* was described well by the hyperbolic model. Fit of the model was also good for rice in competition with *B. frondosa* and *A. indica* (data not shown).

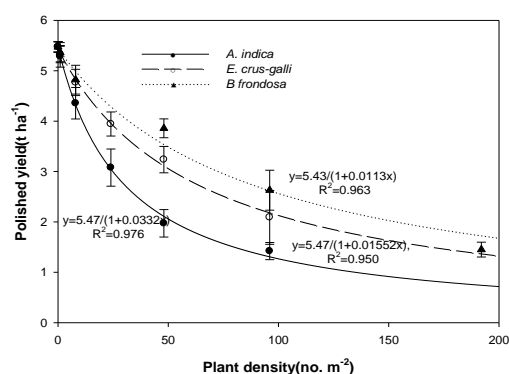


Figure 3. Observed and predicted grain yield of rice as a function of density of *A. indica*, *E. crus-galli*, and *B. frondosa* in Suwon, and Daegu, Korea. The predicted rice grain yield (continuous line) was calculated using (Figure 1.)

Prediction of economic thresholds

Single year economic thresholds of *E. crus-galli*, *B. frondosa* and *A. indica* were calculated by using (Figure 2.) and the parameter estimates in Table 1. The herbicide price to control weeds including the application cost was estimated to be 257.4 US\$ ha⁻¹. The price of grain rice was 2141 US\$ t⁻¹ in 2006. Herbicide efficacy was assumed to be 0.95 regardless of year and herbicide.

Table 1. Parameter estimates and economic threshold (Et) of weeds in machine transplanted rice cultivation.

Weed species	C _a + C _h (\$ ha ⁻¹)	Y _o (t ha ⁻¹)	P (\$ t ⁻¹)	L	H	Et (No m ⁻²)
<i>E. crus-galli</i>	257.4	5.42	2141	0.015	0.95	1.5
<i>A. indica</i>	257.4	5.5	2141	0.032	0.95	0.7
<i>B. frondosa</i>	257.4	5.4	2141	0.011	0.95	2.1

Single year mean economic thresholds (tEs) of *E. crus-galli*, *B. frondosa* and *A. indica* calculated using Cousens' equation were 1.5, 2.5, and 0.7 plants m⁻², respectively.

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EVALUATION OF ALLELOPATHIC ACTIVITY FROM PERUVIAN PLANT SPECIES BY SANDWICH METHOD

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ABSTRACT

Peru is known as country of origin of many cultivated species such as tomatoes, potatoes and peppers. About 20,000 plant species have been reported, many of which are studied for their medicinal properties, but the allelopathic activity of most of them have not been evaluated. In this study a total of 163 species from 60 families of Peruvian plant species collected from the main three natural Peru's regions (the Costa (Coast), Sierra (Highlands) and the Selva (Jungle) region) were screening using the sandwich method. Sandwich method is a bioassay to evaluate the allelopathic activity from leaves, using lettuce like control plant. This screening was tested using 10mg of dry leafs and most of species tested showed inhibition of elongation in lettuce radicle. The species with most highly inhibitory activity (radicle inhibition higher 80%) including 5 families: Asteraceae, Anacardiaceae, Fabaceae, Solanaceae and Bombacaceae.

Keywords: allelopathic activity, Peruvian plants, sandwich method

INTRODUCTION

Located on the west coast of South America, Peru is one of the countries with greatest biodiversity in the world. Peru show three geographic regions; these are the Costa (Coast, Pacific coastline), the Sierra (Highlands, Andean mountains) and the Selva (Jungle, Amazon rainforest), with large variety of ecosystems (Pulgar 1996; Olson & Dinerstein 2002; Brack and Bravo 2005). About 17,000 flowering plants are reported and 27.9% of them are endemic (Brack 1999; Brack & Mendiola 2004; León *et al.* 2006). However, allelopathic properties of these Peruvian plant species have not yet been studied.

The term "allelopathy" describes beneficial and harmful biochemical interaction between organisms; initially for plants, including microorganisms. The secondary metabolites related to this phenomenon are called "allelochemicals"; they are released into the environment by ecological processes of volatilization, leaching, root exudation or by the decomposition of plant residues (Rizvi *et al.* 1992). These compounds have been shown to play important roles in the regulation of plant diversity and in sustainable agriculture (Chang-Hung 1999; Reigosa *et al.* 1999). One of the potential area of applied allelopathy research is for controlling weeds; allelopathic plants can be used as cover, green manure

in cultural practices, into cropping patterns or to developed new compounds to use as natural herbicides (Singh *et al.* 2003; Kráľová 2006; Shennan 2007).

MATERIALS AND METHODS

Plant materials

For this study, a total of 163 species (164 samples) from 60 families of Peruvian plants were screened using the sandwich method under control conditions in the laboratory. Plant materials were collected from the three main regions of Peru: a) 33 species in the Costa, b) 53 species in the Sierra and c) 78 species in the Selva.

a) The Costa; accounts for 12% of land area in Peru. A total of 33 species (20 families) were from this region, collected from the experimental fields at the campus of National Agrarian University La Molina (UNALM), province of Lima, in March, 2006. The samples were mostly native species, including weeds, crops, some medicinal species and cultivated fruits. In addition, leaves of *Phaseolus vulgaris* (cv. Canario) were collected from one-month old plant, after sowing the seeds obtained from local markets in this region.

b) The Sierra; accounts for 28% of land area in Peru. A total of 52 species (19 families) were collected from different places in Ancash Region, of which 31 species (14 families) of native plants were collected from Cajacay District, Bolognesi Province in March, 2006. All these samples were wild plants growing in this region at the end of the rainy season. Additionally, 21 samples (9 families) were of Andean medicinal plants acquired in the market of Huaraz City; these were collected from locations around Huaraz Province in the same season. In addition, *Phaseolus vulgaris* (cv. Ñuña) were collected from one-month old plant, after sowing the seeds obtained from local markets in this region.

c) The Selva; accounts for 60% of land area in Peru. Sampling of Amazonian plants was conducted in 3 locations around Iquitos City, capital of Loreto Region located in Maynas Province, in September, 2007. A total of 78 species of 41 families were collected including: tropical fruit seedlings (9 species) from the Padrecocha Village; tropical fruit trees and Amazonian medicinal plants (11 species) from Peruvian Amazon Research Institute (IIAP); Amazonian medicinal plants and native tropical trees (58 species) from the medicinal garden of the National Reserve Allpahuayo-Mishana.

The samples collected from the Costa and Sierra were identified in the Herbarium of Department of Biology, Faculty of Science at the National Agrarian University La Molina - UNALM. The samples from the Selva were identified by specialists of the Amazonian Medicinal Plants laboratory, at the Peruvian Amazon Research Institute - IIAP.

The leaves of each plant species were collected fresh and placed in a paper bag separately. This material was dried in a drying chamber at 60-70°C for 24 hours. Dried leaves were then stored in closed plastic bags at room temperature until use

Sandwich Method

The sandwich method was developed by Dr. Fujii in Japan (Fujii 1994) as a bioassay to determinate the allelopathic activity of leachate from donor plant leaves, and was employed for the screening of large amounts of medicinal plants, herbal and tree species (Fujii *et al.* 2003, Fujii *et al.* 2004; Morita 2005). A total of 10 mg of dried leaves (or cortex) were placed into 3 wells of a six-well (around 10 cm² area per well) multi-dish plastic plate (35mm×18mm, Thermo Fisher Scientific Inc.). Agar powder (Nacalai Tesque Inc., gelling temperature 30-31 °C) was used as growth medium (0.75% w/v). In each well, 5ml of agar solution was added onto 5ml agar to make two gelatinized layers in between and 5 seeds of the test plant lettuce (*Lactuca sativa* L., Great Lakes No. 366, Takii Co.) were seeded on the surface. The multi-dish was covered with plastic tape, labeled, wrapped in aluminum foil and incubated in the dark at 25°C for 3 days. The length of hypocotyl and radicle of lettuce seedlings were measured on the third day; these data were used to calculate the percent elongation compared to the control.

RESULTS

Most of the screened species belong to the families Asteraceae (25 species), Solanaceae (14 species), Fabaceae (10 species), Amaranthaceae (7 species) and Verbenaceae (7 species).

The seedling radicle elongation in lettuce was inhibited by 162 species (99% of tested species), including 6, 22, 59, 50 and 25 species with 80%, 60-79%, 40-59% 20-39% and 0.3-19% of inhibition respectively, while one species showed no inhibition. For the lettuce hypocotyl, the elongation was inhibited by 55 species (about 34% of tested species). (Figure 1.) shows the distribution of the activity and the species in each region.

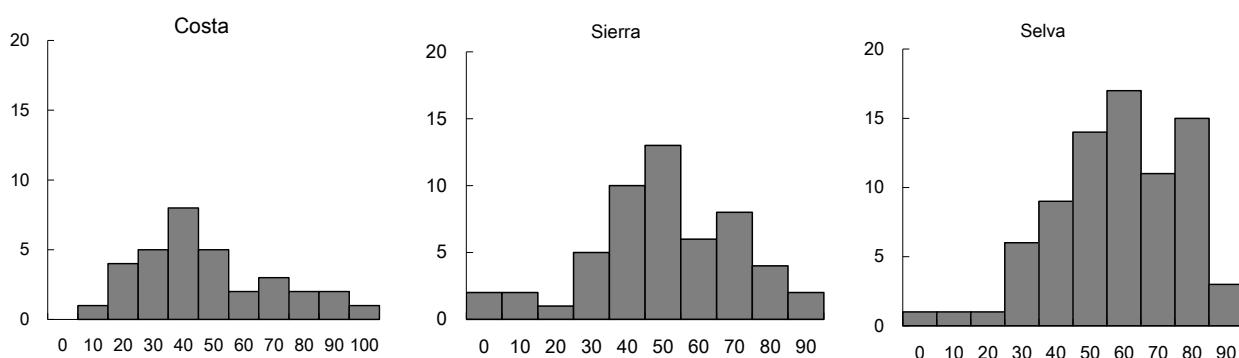


Figure 1. Allelopathic activity and distribution of species in Costa, Sierra and Selva region

DISCUSSION

In this study the species with highest inhibitory activity, referring to the percentage of radicle inhibition higher than 80%, belong to the following 6 species from 5 families; Asteraceae (*Aristeguietia ballii*, *Diplostephium foliosissimum*), Anacardiaceae (*Spondias mombin*), Fabaceae (*Phaseolus vulgaris* cv. Canario and cv. Ñuña), Solanaceae (*Lycopersicon peruvianum*) and Bombacaceae (*Matisia cordata*).

Aristeguietia ballii (Asteraceae) (= *Eupatorium ballii* (King and Robinson 1975)), showed the strongest inhibitory activity and caused 100% growth inhibition in lettuce seedlings. This species is an endemic Peruvian plant growing at a very high altitude (3500- 4000m) in the Central Andes (Beltrán *et al.* 2006). The name of “Asmachilca” is a local name for some species from the genus *Eupatorium* and *Aristeguietia*, and these species are used in traditional medicine for treatment of cough, bronchitis and expectoration.

Spondias mombin (Anacardiaceae) (= *S. lutea*) showed high inhibition of radicle and hypocotyl in lettuce seedlings (97% and 89%, respectively). In this study, a high radicle inhibition (more than 70%) in lettuce seedlings placed with two species of *Spondias* (*S. mombin* and *S. purpurea*) was observed. *S. mombin* is a popular tropical fruit in several countries, and it has been reported for various uses and several medicinal properties (Ayoka *et al.* 2008; Brack 1999).

Diplostephium foliosissimum (Asteraceae) is another medicinal Andean species that grows at high altitude (3200-3300m) (Beltrán *et al.* 2006) and showed high inhibitory activity. It is called “Poleo del Inca” or “Michigato” and is used in folk medicine for treatment of intestinal and gastric pains, and the alcoholic tincture is used to cure hypotension and systemic debilitation (De Feo 2003).

This study was mainly focused on native Peruvian species, collected from different geographical regions and most of them without previous evaluation of their allelopathic activity. Through this screening, strong activity from several species under study was verified; these results show the promising potential of Peruvian species for their use in weed management and for the isolation of bioactive allelochemical compounds.

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EVALUATION OF GRASS HERBICIDE - METAMIFOP ON WEED CONTROL AND PRODUCTIVITY OF DIRECT SEEDED RICE IN TAMIL NADU

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ABSTRACT

Direct seeded rice is subjected to greater weed competition than transplanted rice because both weed and crop seeds emerge at the same time and compete with each other from the germination. Field experiment was conducted during *Kharif* season of 2008-09 and 2009-10 at Tamil Nadu Agricultural University, Coimbatore, India, to evaluate Metamifop for its weed control efficacy and productivity of direct seeded rice. The experiment was laid out in randomized block design with three replications. The treatments comprised of different weed management practices: Metamifop 10 EC at 50, 75, 100 and 125 g a.i. / ha at 2-3 leaf stages, Metamifop 10 EC at 50, 75, 100, 125 and 200 g a.i. / ha at 5-6 leaf stages, Weed free check and Unweeded control. Application of Metamifop 10 EC at 100 and 125 g a.i. / ha at 2 - 3 leaf stages and Metamifop 10 EC at 125 g a.i. / ha at 5 - 6 leaf stages gave comparable results with respect to grass weed control. Between stages of application, application of Metamifop at 2 - 3 leaf stages resulted in better weed control and yield than application at Metamifop 10 EC at 5 - 6 leaf stages. Hence, it could be concluded that the application of Metamifop 10 EC at 100 g a.i. / ha at 2-3 leaf stage was better in controlling grass weeds and recorded higher yield in direct seeded rice.

Key words: Direct seeded rice, Metamifop, Grasses, Weed control efficacy, Yield

INTRODUCTION

Weed infestation continues to be a serious problem in direct seeded rice. Weed growth in direct-seeded rice is severe and is one of the serious limiting factors in realising the yield potential of direct-seeded rice (Rao *et al.*, 2007). The risk of crop yield loss due to competition from weeds by direct seeding methods is higher than for transplanted rice because of the absence of the size differential between the crop and weeds and the suppressive effect of standing water on weed growth at crop establishment (Singh *et al.*, 2007).

Though manual weeding is considered to be the best, the undependable labour availability and escalating wages in many cases has given impetus to the development. Chemical control is the most commonly used and reliable method for controlling weeds in rice. The predominant weed flora found in direct seeded rice was *Echinochloa colonum*, *Cynodon dactylon* and *Dactyloctenium aegyptium* among the grasses. Some of the weeds are not controlled by the traditional herbicides and mostly regenerate after hand weeding. With changing scenario of weed management farmers need herbicides having high efficacy, low phytotoxicity and cost effective as well as no residual effect on succeeding crops.

Introduction of new post-emergence herbicides may help to have a wide spectrum of application time as well as according to weed specificity and may be an effective tool for specific weed management.

Considering these situations, a field experiment investigated the effectiveness of Metamifop 10 EC for controlling grass weeds in direct seeded rice and at the same time, to estimate the influence of herbicide on rice yield.

MATERIALS AND METHODS

The research was conducted in direct seeded rice during *Kharif* season of 2008-09 and 2009-10 at wetlands of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The experimental farm is located at 77°E 11°N latitude 426 m above mean sea level, and the farm receives the normal total annual rainfall of 674.2 mm in 45.8 rainy days. Trial was conducted in soil with fine clay loam type of soil and belonging to *Typic chromusterts* soil group and *noyyal* soil series. This soil is medium in organic carbon content (0.64 per cent) and the available nutrient status is low in nitrogen, medium range of phosphorus and the potassium status is high with neutral to alkaline in soil reaction.

The first crop of rice was planted during *kharif* season 2008-09 (sowing/planting from August to December months) and the second crop during *kharif* season of 2009-10 (sowing / planting from August to October months) with the following treatments:

Treatments		Dose (g a.i. / ha)	Dose (mL / ha)	Time of application
T ₁	Metamifop 10 EC	50	500	2-3 lf stage*
T ₂	Metamifop 10 EC	75	750	2-3 lf stage*
T ₃	Metamifop 10 EC	100	1000	2-3 lf stage*
T ₄	Metamifop 10 EC	125	1250	2-3 lf stage*
T ₅	Metamifop 10 EC	50	500	5-6 lf stage**
T ₆	Metamifop 10 EC	75	750	5-6 lf stage**
T ₇	Metamifop 10 EC	100	1000	5-6 lf stage**
T ₈	Metamifop 10 EC	125	1250	5-6 lf stage**
T ₉	Cyhalofop Butyl 10 EC (Clincher 10 EC)	100	1000	15 DAS
T ₁₀	Weed Free Check	-	-	-
T ₁₁	Untreated Control	-	-	-

*2-3 lf stage of grasses and **5-6 lf stage of grasses

Season	:	<i>Kharif</i> , 2008-09 and <i>Kharif</i> , 2009-10		
Location of the Experiment	:	B- 1 Wetland, Coimbatore, Tamil Nadu, India		
Crop	:	Direct Seeded Rice	Variety	: Co-43
Duration	:	130 days	Plot size	: 25m ²
Spacing	:	20cm X 10cm	Design	: Randomized Block Design
Treatments	:	11	Replications	: 3

RESULTS AND DISCUSSION

Predominant weed flora of the experimental field

The observation made on the common weeds of the experimental field. The experimental field consisted of grasses, sedges and broad leaved weeds (BLW) from unweeded check plot at flowering stage of the crop. The major grass weed was *Echinochloa colona*, *Echinochloa crus-galli* (L.), *Panicum repens* and *Dinebra retroflexa* and the major sedge weed was *Cyperus rotundus* (L.). Among the broad leaved weeds *Eclipta alba* (L.), *Ammania baccifera* (L.) and *Ludwigia parviflora* (L.) were the dominant species.

Weed control efficacy

In both crops, among the post-emergence herbicide application, Metamifop 10 EC at 125 g a.i. / ha at 2 - 3 leaf stage (T₄) recorded lower grass weed density and it was comparable with Metamifop 10 EC at 100 g a.i. / ha (T₃) and Metamifop 10 EC at 125 g a.i. / ha at 5 - 6 leaf stages (T₈) on 30 DAS (Table 1). The above said treatments reigned superior in weed control than the standard Cyhalofop butyl 10 EC at 100 g a.i. / ha.

At 60 DAS, application of Metamifop at 125 g a.i. / ha at 2-3 leaf stage (T₄) was very effective in controlling the density of grasses and it was followed by Metamifop 10 EC at 100 g ai / ha at 2-3 leaf stage (T₃) and Metamifop 10 EC at 125 g a.i. / ha at 5 - 6 leaf stages (T₈) which recorded lower weed densities. Metamifop at the increased dose of Metamifop from 50 to 125 g a.i. / ha lowered the density of grasses considerably as compared to unweeded control (T₁₂). Between stages of herbicide application, use of Metamifop at 2-3 leaf stage gave better control of grasses than 5-6 leaf stage.

The weed free check (T₁₁) recorded the lower weed density 30 and 60 DAS during both the seasons. Unweeded control recorded higher total weed density at all stages. The control of the grasses by the Metamifop 10 EC treatments and the standard Cyhalofop butyl 10 EC has shown the corresponding similar trend in the total weed density (Table 1).

Table 1. Effect of Metamifop 10 EC on grass weed density and total weed density in direct seeded rice

Treatments	Grass weed density (m ⁻²)*		Total weed density (m ⁻²)*	
	<i>Kharif</i> , 2008-09	<i>Kharif</i> , 2009-10	<i>Kharif</i> , 2008-09	<i>Kharif</i> , 2009-10

	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
T ₁	3.21 (22.7)	3.46 (29.89)	3.43 (29.2)	3.60 (33.3)	4.19 (63.81)	4.48 (86.0)	4.39 (79.15)	4.67 (105.0)
T ₂	2.68 (12.6)	2.98 (17.77)	3.0 (18.1)	3.30 (26.2)	4.07 (56.90)	4.37 (77.3)	4.25 (68.03)	4.59 (96.9)
T ₃	1.63 (3.1)	1.77 (3.89)	1.96 (5.4)	2.20 (6.7)	3.93 (48.80)	4.30 (71.8)	4.17 (63.02)	4.49 (87.4)
T ₄	1.59 (2.9)	1.76 (3.80)	1.56 (2.8)	1.90 (5.0)	3.91 (47.80)	4.26 (69.0)	4.11 (59.41)	4.41 (80.7)
T ₅	3.30 (25.0)	3.69 (38.13)	3.56 (33.3)	4.00 (52.4)	4.21 (65.08)	4.57 (94.6)	4.44 (83.24)	4.83 (123.1)
T ₆	3.21 (22.7)	3.38 (27.50)	3.62 (35.3)	3.90 (48.3)	4.14 (61.0)	4.44 (82.8)	4.46 (85.27)	4.79 (119.0)
T ₇	2.79 (14.3)	3.00 (18.13)	2.82 (15.6)	3.20 (21.8)	4.07 (56.33)	4.39 (78.6)	4.30 (72.21)	4.57 (94.60)
T ₈	1.69 (3.4)	1.75 (3.73)	1.73 (3.7)	2.20 (6.60)	3.94 (49.41)	4.25 (68.4)	4.13 (60.28)	4.40 (80.7)
T ₉	2.64 (12.1)	2.97 (17.53)	2.68 (12.6)	3.30 (25.7)	4.05 (55.43)	4.40 (79.2)	4.26 (69.14)	4.59 (96.4)
T ₁₀	1.33 (1.8)	1.43 (2.20)	1.29 (1.6)	1.50 (2.30)	1.67 (3.30)	1.70 (3.5)	1.51 (2.55)	1.82 (4.2)
T ₁₁	4.30 (71.5)	4.58 (95.23)	4.30 (72.3)	4.50 (91.70)	4.74 (112.50)	5.06 (115.7)	4.85 (126.55)	5.10 (162.4)
CD (P=0.05)	0.067	0.068	0.29	0.17	0.14	0.10	0.12	0.18

T₁ to T₄ - Application at 2-3 leaf stage of grass weeds; T₅ to T₈ - Application at 5 - 6 leaf stage of grass weeds

Figures in parenthesis are means of original values; * Data are square root transformed values

Weed free check (T₁₀) recorded the lowest grass weed dry weight 30 and 60 DAS in both crops. Among the herbicide treatments, lower grass weed dry weight was recorded with application of Metamifop 10 EC at 125 g a.i. / ha at 2 - 3 leaf stage (T₄) followed by Metamifop 10 EC at 100 g a.i. / ha at 2 - 3 leaf stage (T₃) and Metamifop 10 EC at 125 g a.i. / ha at 5-6 leaf stage (T₈) which were comparable with each other (Table 2).

The same trend was observed at 60 DAS with regard to grass weed dry weight. Unweeded control (T₁₁) recorded higher grass weed dry weight at 30 and 60 DAS.

Table 2. Effect of Metamifop 10 EC on grass weed dry weight and total weed dry weight in direct seeded rice

Treatments	Grass weed dry weight* (g/m ²)				Total weed dry weight * (g/m ²)			
	<i>Kharif, 2008-09</i>		<i>Kharif, 2009-10</i>		<i>Kharif, 2008-09</i>		<i>Kharif, 2009-10</i>	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
T ₁	3.90 (47.7)	4.63 (100.2)	4.15 (61.3)	4.66 (103.7)	4.91 (134.0)	5.40 (219.5)	5.12 (166.2)	5.84 (340.7)

T ₂	3.17 (21.9)	4.13 (60.5)	3.64 (36.2)	4.51 (89.1)	4.69 (107.2)	5.21 (181.4)	4.92 (136.1)	5.69 (296.4)
T ₃	2.20 (7.1)	2.66 (12.4)	2.22 (7.2)	3.09 (20.8)	4.55 (92.7)	5.12 (165.1)	4.80 (119.7)	5.58 (264.4)
T ₄	2.09 (6.1)	2.53 (10.5)	1.97 (5.3)	2.94 (17.1)	4.52 (90.3)	5.08 (158.5)	4.73 (112.3)	5.52 (250.2)
T ₅	4.13 (59.9)	2.84 (125.0)	4.38 (77.9)	5.16 (171.9)	5.04 (152.6)	5.47 (234.6)	5.28 (194.8)	6.00 (403.9)
T ₆	4.05 (55.6)	4.52 (90.6)	4.48 (86.5)	5.08 (158.7)	5.02 (149.4)	5.34 (206.3)	5.35 (208.7)	6.01 (404.8)
T ₇	3.30 (25.1)	4.15 (61.3)	3.28 (25.9)	4.28 (70.8)	4.65 (103.2)	5.21 (181.2)	4.79 (119.9)	5.70 (296.0)
T ₈	2.15 (6.6)	2.63 (12.0)	2.19 (6.9)	3.15 (21.4)	4.56 (93.3)	5.13 (167.7)	4.75 (113.9)	5.57 (262.2)
T ₉	3.33 (26.1)	4.25 (67.9)	3.29 (24.8)	4.47 (85.8)	4.74 (112.3)	5.40 (220.5)	4.86 (126.5)	5.76 (315.5)
T ₁₀	1.70 (3.5)	2.18 (6.8)	1.64 (3.2)	2.21 (7.1)	2.13 (6.40)	2.31 (8.10)	1.94 (5.0)	2.71 (13.2)
T ₁₁	5.25 (118.8)	5.65 (284.6)	5.26 (190.8)	5.62 (274.2)	5.70 (297.0)	5.84 (341.0)	5.81 (334.1)	6.19 (485.7)
CD (P=0.05)	0.12	0.14	0.26	0.22	0.12	0.10	0.15	0.21

T₁ to T₄ - Application at 2-3 leaf stage of grass weeds; T₅ to T₈ - Application at 5 - 6 leaf stage of grass weeds

Figures in parenthesis are means of original values; * Data are square root transformed values

Total weed dry weight

Weed free check (T₁₁) registered lower total weed dry weight at 30 and 60 DAS, during both the seasons. The control of the grasses by the Metamifop 10 EC treatments and the standard Cyhalofop butyl 10 EC has shown the corresponding similar trend of both grasses dry weight and the total weed dry weight both at 30 and 60 DAS was observed during *Kharif* 2008-09.

During *Kharif* 2009-10, at 30 and 60 DAS, lower total weed dry weight was recorded with application of Metamifop 10 EC at 125 g a.i. / ha at 2-3 leaf stage (T₄) followed by Metamifop 10 EC at 100 g a.i. / ha at 2 - 3 leaf stage (T₃) and Metamifop 10 EC at 125 g a.i. / ha at 5-6 leaf stage (T₈), which remained comparable. Unweeded control (T₁₁) recorded higher total weed dry weight at 30 and 60 DAS during both the seasons (Table 2).

Table 3. Effect of Metamifop 10 EC on weed control efficiency in direct seeded rice

Treatments	Weed Control Efficiency (%)			
	<i>Kharif</i> , 2008-09		<i>Kharif</i> , 2009-10	
	30 DAS	60 DAS	30 DAS	60 DAS
T ₁ . Metamifop 10 EC at 50 g ai ha ⁻¹	74.8	64.8	67.9	62.17
T ₂ . Metamifop 10 EC at 75 g ai ha ⁻¹	88.4	78.7	81.0	67.50
T ₃ . Metamifop 10 EC at 100 g ai ha ⁻¹	96.3	95.7	96.2	92.43

T ₄ - Metamifop 10 EC at 125 g ai ha ⁻¹	96.8	96.3	97.2	93.78
T ₅ - Metamifop 10 EC at 50 g ai ha ⁻¹	68.3	56.1	59.2	37.32
T ₆ - Metamifop 10 EC at 75 g ai ha ⁻¹	70.6	68.2	54.6	42.11
T ₇ - Metamifop 10 EC at 100 g ai ha ⁻¹	86.7	78.4	86.4	74.18
T ₈ - Metamifop 10 EC at 125 g ai ha ⁻¹	96.5	95.8	96.4	92.18
T ₉ - Cyhalofop butyl 10EC at 100 g ai ha ⁻¹ @ 15 DAS	86.2	76.2	87.0	68.70
T ₁₀ - Weed free check	98.1	97.6	98.3	97.40
T ₁₁ - Unweeded control	0.0	0.0	0.0	0.0

T₁ to T₄ - Application at 2-3 leaf stage of grass weeds

T₅ to T₈ - Application at 5 - 6 leaf stage of grass weeds

Weed Control Efficiency (WCE %)

During both the seasons, weed free check (T₁₀) registered higher weed control efficiency (Table 3). At 30 and 60 DAS, higher weed control efficiency was recorded with application of Metamifop 10 EC at 125 g a.i. / ha at 2-3 leaf stage (T₄) followed by Metamifop 10 EC at 100 g a.i. / ha at 2 - 3 leaf stage (T₃) and Metamifop 10 EC at 125 g a.i. / ha at 5-6 leaf stage (T₈) which remained comparable with each other. The above three treatments gave significantly higher weed control efficiency than the standard Cyhalofop butyl 10 EC at 100 g a.i. / ha.

Yield attributes

Number of productive tillers per plant and test weight of grains was recorded. During both the seasons, significantly, higher number of productive tillers was recorded with weed free check (T₁₀) which remained on par with Metamifop 10 EC at 125 g a.i. / ha at 2-3 leaf stage (T₄). This was followed by Metamifop 10 EC at 125 g a.i. / ha at 5-6 leaf stage (T₈) and Metamifop 10 EC at 100 g a.i. / ha at 2 - 3 leaf stage (T₃) treatments (Table 4). Lower number of productive tillers was observed in unweeded control. There was no significant difference in the test weight (1000 grain weight) of grains observed between the treatments.

Table 4. Effect of Metamifop 10 EC on productive tillers (m⁻²), test weight (g/1000 grains) and yield (kg/ha) in direct seeded rice

Treatments	Yield attributes				Yield (kg/ha)			
	Kharif, 2008-09		Kharif, 2009-10		Kharif, 2008-09		Kharif, 2009-10	
	Productive tillers	Test weight	Productive tillers	Test weight	Grain yield	Straw yield	Grain yield	Straw yield
T ₁	435	16.8	419	18.2	3153	4607	3017	4028
T ₂	453	17.2	441	17.6	3725	5325	3510	4261

T ₃	465	17.5	509	17.9	4124	5661	4280	5217
T ₄	535	17.8	505	17.5	4328	5855	4401	5363
T ₅	434	16.5	431	18.0	2985	4593	2932	3898
T ₆	465	16.4	449	18.1	3121	4732	3148	3877
T ₇	446	16.8	435	17.4	3860	5282	3560	4390
T ₈	475	17.2	503	17.4	4255	5627	4362	5543
T ₉	450	16.2	44	17.7	3728	5182	3667	4810
T ₁₀	497	17.3	551	18.2	4857	6228	4858	5975
T ₁₁	373	15.8	362	17.0	2060	3306	2330	3254
CD (P=0.05)	56.6	NS	37	NS	345	311	277	503

T₁ to T₄ - Application at 2-3 leaf stage of grass weeds; T₅ to T₈ - Application at 5 - 6 leaf stage of grass weeds; Figures in parenthesis are means of original values

Grain and Straw yield

The weed free check (T₁₀) recorded significantly the higher grain and straw yields. Among the post-emergence herbicide application, Metamifop 10 EC at 125 g a.i. / ha at 2 - 3 leaf stage (T₄) recorded higher grain and straw yields and the same treatment (T₄) was on par with Metamifop 10 EC at 100 g a.i. / ha at 2 - 3 leaf stage (T₃) and Metamifop 10 EC at 125 g a.i. / ha at 5 - 6 leaf stage (T₈) (Table 4). The above said treatments resulted in significantly higher grain yield than the standard Cyhalofop butyl at 100 g a.i. / ha. Lower doses of Metamifop 10 EC at 50 g a.i. / ha at 2 - 3 leaf stage (or) 5 - 6 leaf stage (T₁ and T₅) gave lower grain and straw yield compared to other herbicidal treatments during both the years.

DISCUSSION

The major grass weeds were *Echinochloa colona*, *Echinochloa crus-galli* (L.), *Panicum repens*. Pacanoski and Glatkova (2009) also reported that the most prevailing weeds in direct seeded rice were *Cyperus rotundus*, *Echinochloa crus-galli* and *Heteranthera limosa*.

The weed free check recorded significantly the lowest grass weed density and higher weed control efficiency in all crop stages. Unweeded control recorded higher grass weed density and lower weed control efficiency (Mohan *et al.*, 2005). Application of Metamifop 10 EC at 100 and 125 g a.i. / ha at 2 - 3 leaf stages and Metamifop 10 EC at 125 g a.i. / ha at 5 - 6 leaf stages gave comparable results with respect to grass weed control of rice and has performed significantly better than the standard Cyhalofop butyl 10 EC at 100 g a.i. / ha. A mixture of mefenacet and bensulfuron-methyl (Mefenacet 53 WP) showed the reduced control of *Echinochloa crus-galli* and *Heteranthera limosa* in both localities although mefenacet is mainly active against grass weeds (Hess *et al.* 1990). Yield attributes and yield also higher with the application of Metamifop 10 EC at 100 and 125 g a.i. / ha at 2 - 3 leaf stages and Metamifop 10 EC at 125 g a.i. / ha at 5 - 6 leaf stages

when compared to other treatments. As earlier reported by Rao *et al.*, (2008), pre-emergence application of pendimethalin 1.0 kg / ha super imposed with one hand weeding at 30 DAS was effective in reducing weed growth and increased grain yield of direct-seeded semi dry rice.

Between stages of application, application of Metamifop at 2 - 3 leaf stages resulted in better weed control and yield than application at Metamifop 10 EC at 5 - 6 leaf stages. Excellent control of *Echinochloa crus-galli* with penoxulam applied at the three-to-four leaf growth stage was reported by Ottis *et al.* (2003). Hence, it could be concluded that the application of Metamifop 10 EC at 100 g a.i. / ha at 2-3 leaf stage was found to be better in controlling grass weeds in direct seeded rice.

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PLANNING FOR EFFECTIVE WEED MANAGEMENT: LESSONS FROM SRI LANKA

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ABSTRACT

In tropical Sri Lanka, Water Hyacinth [*Eichhornia crassipes* (Mart.) Solms.] and Salvinia (*Salvinia molesta* D. S. Mitchell) continue to dominate eutrophic waterways in both rural and urban environments. In addition, several other highly invasive species have also recently become problems in waterways, and these include: Alligator Weed [*Alternanthera philoxeroides* (Mart.) Griseb], Water Spinach *Ipomoea aquatica* Forssk. and Hydrilla [*Hydrilla verticillata* (L. f.) Royle]. Infestations of Primrose Willows - *Ludwigia peruviana* (L.) Hara and *L. octovalvis* are increasing in abundance in reclaimed marshlands and drainage canals, while several others (e.g. *L. decurrens* and *L. hyssopifolia*) continue to be major weed problems in rice agriculture.

Relatively recent introductions of major global weeds (i.e. Giant Mimosa – *Mimosa pigra* L.; Parthenium - *Parthenium hysterophorus* L.; *Austro eupatorium inulifolium* (Kunth) R.M. King & H. Rob.) highlight Sri Lanka's problems. Their pattern of spread, via major roads, is attributable to accelerated development of infrastructure and use of contaminated machinery. Two recent escapees from aquarium industry: *Mayaca fluviatilis* Aubl. and *Ludwigia sedioides* L. are now spreading, at least in one district.

Sri Lanka has a poor record of managing weeds, largely due to inadequate mechanisms to deal with them early, or prevent their entry through border protection. Recent stakeholder consultations identified insufficient funding for on-ground works and for research; and the absence of a central coordination mechanism, as major constraints. Control options are also limited (i.e. limited array of herbicides, or bio-control agents) and experience is also lacking in implementing large-scale integrated weed management programs. To effectively tackle invasive species in Sri Lanka, a *National Weed Strategy* (NWS) has been developed. This national framework aims to provide increased weed science education at tertiary level and increased training for government officials and farmers. It also aims provide a mechanism for efficient information sharing and effective multiple-stakeholder cooperation and participation in managing weeds across landscapes.

Keywords: Aquatic weeds, Sri Lanka, National Weed Strategy

INTRODUCTION

The agriculture sector in Sri Lanka provides livelihood for around 35% of the country's work force and contributes around 19% of GDP. This sector comprises: food crops (e.g. rice, pulse crops and vegetables), plantation crops (Tea, Rubber and Coconut) and export crops (Pepper, Cloves and Nutmeg etc.) and. Weeds are a major constraint to achieving high productivity and self-sufficiency in agriculture, and in some cases, weeds reduce crop yields (quantity) by as much as 50%. Weeds also reduce the quality of crop produce, thereby reducing the overall productivity of land and farmers' income from agriculture. Weeds reduce the quality the island's uniquely native biodiversity and have many negative impacts on the health of waterways and aquatic resources (Amarasinghe and Marambe, 1997; Bambaradeniya et al., 2001; Marambe, et al., 2001; MFE, 1999).

Until the recent centuries and colonial interventions, the natural barriers of the ocean provided the isolation that was essential to the evolution of Sri Lanka's unique species and ecosystems. In less than two centuries, these barriers have been rendered ineffective, because of an increased global trade that enables weed species to travel vast distances. Invasion by new alien species, and expanding infestations of existing invasives, are both major threats to biological diversity, second only to habitat degradation and losses, which occurs as a result of increasing population pressure in countries like Sri Lanka.

Sri Lanka (earlier known as 'Ceylon') is an ancient hydrological, rice-based civilization, dating back to more than 2500 years. During the last few centuries of colonial rule, plantation-scale agriculture took hold in the island – firstly, coffee and tea, and then, rubber, coconut and cinnamon plantations. Such a historical background provided Sri Lankan farmers with a sound tactical knowledge of how to manage weeds. Nevertheless, the new threats posed by a host of invasive species, and their increased abundance, have put pressures on the island's biodiversity and agricultural productivity. In the fight against weeds, a nation-wide planning framework is now required to coordinate and enhance the weed management efforts of the community, industry and government.

During the past three years, or so, Sri Lanka Council for Agricultural Research Policy (CARP) gave leadership to develop a *National Weed Strategy* (NWS), following a 'whole-of-government' approach, with input from government departments, research institutes, Universities, industry and the community. The objective of this paper is to present an overview of this national approach undertaken in Sri Lanka, and to discuss some issues that may enlighten other Asia-Pacific countries, which are tackling similar problems.

Major weeds of concern

Sixteen weeds of 'National Significance' have been identified (Table 1), based on the scientific information available and the research by Departments of Agriculture and Export Agriculture, Research Institutes (Tea, Rubber, Coconut, Sugarcane), and Universities. The list includes major weeds of rice agriculture, waterways and plantation crops.

As an ancient hydrological civilization, the island is dotted with thousands of inland lakes (or 'tanks'), which furnish water, primarily for a rice-based agriculture. These lakes and major rivers are inter-connected by irrigation networks, some of which are ancient. Inadequate management of waterways, over the years, has led to sedimentation and

significant aquatic weed problems. Aquatic weeds reduce the flow of water, leading to inefficient irrigation. They reduce the habitat for aquaculture, cause water loss through evaporation; and provide breeding grounds for mosquitoes, including malaria and dengue.

Major aquatic weeds of Sri Lanka include Water Hyacinth, *Salvinia*, *Ipomoea aquatica* Forssk., *Hydrilla* [*Hydrilla verticillata* (L. f.) Royle] and *Myriophyllum* L. spp. Infestations of Primrose Willows - *Ludwigia peruviana* (L.) Hara and *L. octovalvis* (Jacq.) Raven, are dominant in abandoned rice-fields, reclaimed marshlands and drainage canals, while *L. decurrens* Walt. and *L. hyssopifolia* (G. Don) Exell, are major rice-field weeds. Other major rice-field weeds in Sri Lanka include grasses, such as *Echinochloa crusgalli*; *Ischaemum rugosum* Salisb., *Isachne globosa* (Thunb.) O. Ktze and *Leptochloa chinensis*; various sedges, such as *Cyperus iria* L.; *C. haspan* L., *Fimbristylis miliacea* Vahl.; and many broad-leaved species, including Primrose Willows (Chandrasena, 1990).

New agricultural and environmental weed problems that are causing great concern in Sri Lanka include various kinds of 'Weedy Rice' (which are hybrids), Alligator Weed (*Alternanthera philoxeroides*), *Mimosa pigra*, *Parthenium hysterophorus* (Bambaradeniya et al., 2001; Marambe, et al., 2001) and *Austro eupatorium inulifolium*. Among new aquatic weed threats, two escapees from the aquarium trade - *Mayaca fluviatilis* and *Ludwigia sedioides* have now spread at least in one district (Yakandawala and Yakandawal, 2007).

Table 1. Sri Lanka's Weeds of National Significance (Source: CARP, 2008)

Common Name	Botanical Name	Habitat/Crop affected most
Madeira Vine	1. <i>Anredera cordifolia</i> (Ten.) Steenis	Tea plantations
Siam Weed	2. <i>Chromolaena odorata</i> (L.) King & Robbins	Coconut, Cinnamon plantations
Dodder	3. <i>Cuscuta campestris</i> Yunck.	All plantations; derelict lands
Purple Nutsedge	4. <i>Cyperus rotundus</i> L.	Rice agro-ecosystem
Barnyard Grass	5. <i>Echinochloa crusgalli</i> (L.) Beauv	Rice agro-ecosystem
Water Hyacinth	6. <i>Eichhornia crassipes</i> (Mart.) Solms	Irrigation tanks; drainage networks
Cogongrass	7. <i>Imperata cylindrica</i> (L.) Beauv	Tea plantations
Lantana	8. <i>Lantana camara</i> L.	National parks; derelict lands
Red Sprangletop	9. <i>Leptochloa chinensis</i> (L.) Nees	Rice agro-ecosystem
Chinese Creeper	10. <i>Mikania micrantha</i> Kunth	Rubber plantations
Giant Mimosa	11. <i>Mimosa pigra</i> L.	Riverbanks
Congress Weed	12. <i>Parthenium hysterophorus</i> L.	Fallow fields, marshy areas
Guinea Grass	13. <i>Panicum maximum</i> Jacq.	National parks; roadsides
Mission Grass	14. <i>Pennisetum polystachyon</i> (L.) Schult.	Roadsides; national parks
Giant Salvinia	15. <i>Salvinia molesta</i> D.S. Mitchell	Irrigation tanks, network; rice-fields
Getakola	16. <i>Spermacoce hispida</i> L.	Tea, rubber, coconut, plantations

Sri Lanka's national weed strategy

The Sri Lanka *National Weeds Strategy* (NWS) 2009-2014 was developed as a 5-year planning framework for improved management of weeds across the country. This strategy, largely based on the Australian model, has three broad goals and several objectives under each goal (Table 2). The primary goals are to: (1) Prevent introduction, entry and spread of new plant species with weed potential; (2) Reduce the impact of weeds of national significance by effectively implementing management programs for existing weed problems; and (3) Provide the framework for capacity building in weed management.

Sri Lanka's NWS is, of necessity, a broad, over-arching document. Assigning roles and responsibilities, the NWS establishes a nation-wide planning and implementation framework to give direction to weed management efforts of governmental agencies, community, industry and individuals. It is seen as the first step towards achieving consensus between often fragmented weed management efforts. When properly funded and implemented it will co-ordinate action to effectively and sustainably undertake weed management throughout Sri Lanka. It will also drive the required capacity building to meet future threats of weeds, as an adaptation response to potential, future climate change.

Table 2. Summary of Goals, Objective, Strategies and Proposed Actions - Sri Lanka's National Weed Strategy

Goal	Objectives	Strategies	
1. Prevent outbreaks of new weed problems	1.1 Prevent introductions, entry and spread of new plant species with weed potential	1.1A Develop inventories of plant species with weedy potential	1.1A1 Produce a List/Book of major noxious weeds in Sri Lanka with photos/descriptions. Include those of regional/global significance, relevant to Sri Lanka
			1.1A2 Establish databases and links to international databases (e.g GISP)
			1.1A3 Review procedures and constraints for implementing quarantine regulations in all ports of entry; identify training needs
		1.1B Ensure effective enforcement of plant quarantine regulations	1.1B1 Develop the list of declared/quarantined weeds
			1.1B2 Record all successful cases of implementing procedures
			1.1B3.1 Conduct occasional surveillance surveys of 'Hot Spots' of entry by agricultural sector and or Nursery Trade, Aquarium Industry, Boat and Fishing crafts, etc
			1.1B3.2 NPQS to develop list of locations and/or activities of 'risk'
			1.1B3.3 Develop operational plan for surveillance and inspections of 'Hot Spots' and targeted trades/activities
			1.1B4.1 Develop resource materials for plant quarantine (Herbarium; Seed photographic bank)
			1.1B4.2 NPQS to develop resources required to upgrade plant quarantine laboratory and services
		1.1C Maintain a 'Critical Mass' of trained Quarantine Staff	1.1C1 NPQS to invest in increasing capacity by training more people; undertake succession planning
			1.1C2 Obtain training in Border Protection with donor assistance. Conduct internal training to build capacity within 2 years
		1.1D Improving awareness among people at all levels	1.1D1 Conduct seminars to policy makers, administrators, practitioners and scientists
			1.1D2 Organise Public Seminar/Conference on importance of plant quarantine and border protection
			1.1D3 Conduct campaigns through mass media.
			1.1D4 Develop fact sheets on potential noxious weeds, based on global models (relevant to SL)
	1.2 To ensure early detection of and rapid action against new weed problems	1.2A Develop an effective reporting system	1.2A1 Undertake at least 2 surveys per year, target specific vulnerable landscapes (i.e. road network, waterways, wetlands, national parks, World Heritage Areas)
			1.2A2 Develop reporting form and make available to all stakeholders
		1.2B Promote Public Participation in weed surveys	1.2B1 Develop public campaign using NGOs
			1.2B2 Train 'Weed Warriors'; volunteers to undertake weed surveys, target vulnerable landscapes

- and reporting
- 1.2C To reduce weed spread to new areas in the country
- 1.2B3 Publish information available on new weed incidence
- 1.2B4 Train Staff of various Governmental and NGO through Workshops/Seminars
- 1.2C1 Conduct Workshop on 'Vectors of Weed Spread' and how to manage them; develop guidelines
- 1.2C2 Review local legislation required strengthening local management with various authorities.
- 1.2C3 Train Staff of various Governmental and Non-Governmental Organizations/agencies through one-day Workshops/Seminars

Table 2 (continued). Summary of Goals, Objective, Strategies and Proposed Actions - Sri Lanka's National Weed Strategy

2. To reduce the impact of weeds of national significance (WONS)	2.1 To facilitate identification of WONS on agricultural and non-agricultural land	2.1A Establish criteria for determining weeds of significance to Sri Lanka	2.1A1 Develop guidelines / procedures to establish WONS.
			2.1A1 Produce a List/ Book of major noxious weeds found in Sri Lanka with photos/descriptions. Include those of regional and global significance, relevant to Sri Lanka.
			2.1A3 Develop AV materials on weed identification
			2.1A4 Develop and host dedicated Web Site to achieve publicity, accessible by all stakeholders
	2.2 To apply integrated and cost effective weed management techniques to reduce impact of established weeds	2.2A Encourage research on weed biology, ecology and control through Integrated Weed Management (IWM)	2.2A1 Prioritise weed management research at national level, especially through biological control
			2.2A2 Implement IWM research at national level combining all available control technologies
			2.2A3 Organize a Herbicide Technology Workshop to update knowledge and address issues
			2.2A4 Organize training on IWM Implementation
		2.2B Explore possibilities for utilization of weeds	2.2A5 Promote collaborative research proposals for funding.
			2.2A6 Establish links with international research institutes / universities.
			2.2B1 Promote utilization as a method of management
			2.2B2 Review opportunities and promote utilization of weeds as a management tool
3. Provide the framework & capacity for the coordinated management of WONS	3.1 To strengthen national R&D, education and training capacity for cost effective & sustainable weed management	2.2C Improve community awareness at all levels	2.2B3 Establish research links with international research institutes / universities.
			2.2C1 Conduct seminars to policy makers, administrators, practitioners and scientists.
			2.2C2 Conduct a campaign through mass media.
		3.1A Capacity Building	3.1A1 Identify training and manpower needs of the sector with assistance of stakeholders.
			3.1A2 Assist training institutes to offer courses (e.g Diplomas, M.Sc. Courses in Management of Biodiversity, Landscapes, Aquatic Resources, Wildlife, Plantation Management)
			3.1A2 Provide assistance to distance training institutes (i.e. the Open University) – to offer courses related vegetation/weed management
			3.1A4 Encourage various agencies/Institutes to review their weed management mandate and operational needs

3.2 To encourage the development & implementation of strategic plans for weed management at all levels	3.1B Provide financial assistance for weed management R&D and for targeted projects	3.1B1 Apply for Funding (national/ international; governmental/ private sector) using suitable models
	3.2A Develop a coordinated mechanism for implementation of weed management strategies.	3.2A1 Establish focal points and contacts with coordinating agency 3.2A2 Establish NWS Newsletter and other promotional material to promote integration of efforts and coordinate responses 3.2A3 Undertake annual review of weed problems and management activities.
	3.2B Define the role of stakeholders' in weed management programmes	3.2B1 Develop Policy position and Discussion paper with multiple stakeholders 3.2B2 Annual evaluation of all weed management activities and progress in all sectors 3.2C1 Develop accreditation system; Recognize training as part of career progression

Planning for effective weed management – some principles

The 'model of facilitated participation', promoted by the NWS, encourages all stakeholders to work together to achieve more effective management of existing weeds, and to limit future introduction of new weed species. Implementation responsibilities for the NWS and linkages to other programs (such as Biodiversity management) are still being developed.

However, the success of a national framework depends on engaging multiple stakeholders diligently, efficiently and transparently. Stakeholders for the NWS include: Ministries of Agriculture, Agrarian Services, Environment, Plantation Industries, and various Departments and Agencies, which come under those Ministries. An expanded list would include: Departments of Agriculture; Forestry; Wildlife; Irrigation; Water Resources; National Herbarium; Central Environment Authority (CEA); Mahaweli Authority, Commodity Research Institutes (e.g. Tea, Rubber, Coconut, Sugar Cane, Minor Export Crops), National Aquatic Resources Authority (NARA), University Faculties of Agriculture and Science, other training institutes, funding and policy Institutes (such as CARP) and the private sector (e.g. Plantation Industries; Agrochemical Industries).

These stakeholder groups participated in a UNDP funded workshop forum in December 2009, to fine tune the NWS and further discuss what approaches might work and how. The outcomes of the consultations, summarised below, provide insights on the planning that would be required to implement elements of the NWS.

- Emphasis on existing local knowledge – To effectively manage the threat posed by weeds, and to empower farmers and communities, it is necessary to integrate existing 'tactical' knowledge of local farmers with other resources and novel approaches.
- Prioritizing and rationalizing resources - Developing countries do not have unlimited resources that can be directed towards weed control. Therefore, it is essential to prioritise and rationalise resources. This can be achieved by identifying which species is the fastest growing, most disruptive, and most likely to have adverse impacts. The prioritized list (Table 1) reflects the current stakeholder concerns on each weed's present economic and environmental impact, or future potential for harmful impacts.
- Prevention is essential - To minimize weed problems from arising, and later becoming entrenched, it is essential to focus on 'pathways of entry' and 'vehicles of spread' of invasive species. As targeted by the NWS Goal 1 (Table 2), actions include strengthening border protection and quarantine services, focusing on imports, shipping containers; airfreight; horticulture, aquarium industries and other agri-businesses.
- Not all weeds are bad all the time - It is evident that some weeds may be tenacious and potentially harmful. However, Sri Lanka's ancient culture is strongly linked with recognising the high value of plants as biological resources – for food, fodder, shelter, and for medicinal values. The renowned 'aruyvedic' (eastern) medicine system uses a large number of colonising plants as medicines. Therefore, our philosophy should be to identify and manage only those species, which threaten the quality of our natural and productive landscapes, but not to malign all 'colonising plants' as 'bad' all the time. Ideally, a national approach to integrated weed management should also exploit any opportunities for utilization of invasive species for human benefits.

- Ecologically-based, economically-feasible weed management – Agriculture is constantly under pressure to increase crop production, due to high demand for food and reduced availability of land. This pressure compels farmers to place a high reliance on herbicides as ‘quick and easy’, solutions to weed problems. Rational use of herbicides will continue to be important to manage some of the more pernicious species. However, a sustainable future will be one that develops practices that would ‘integrate’ herbicides with other ecologically-friendly approaches. To do this, it is essential to follow principles of ‘ecologically-based’ weed management, and manipulate critical components of the weed-crop interactions in the field to favour crop growth over that of weeds. As depicted in Figure 1, there are many tactical avenues by which weed growth can be discouraged.

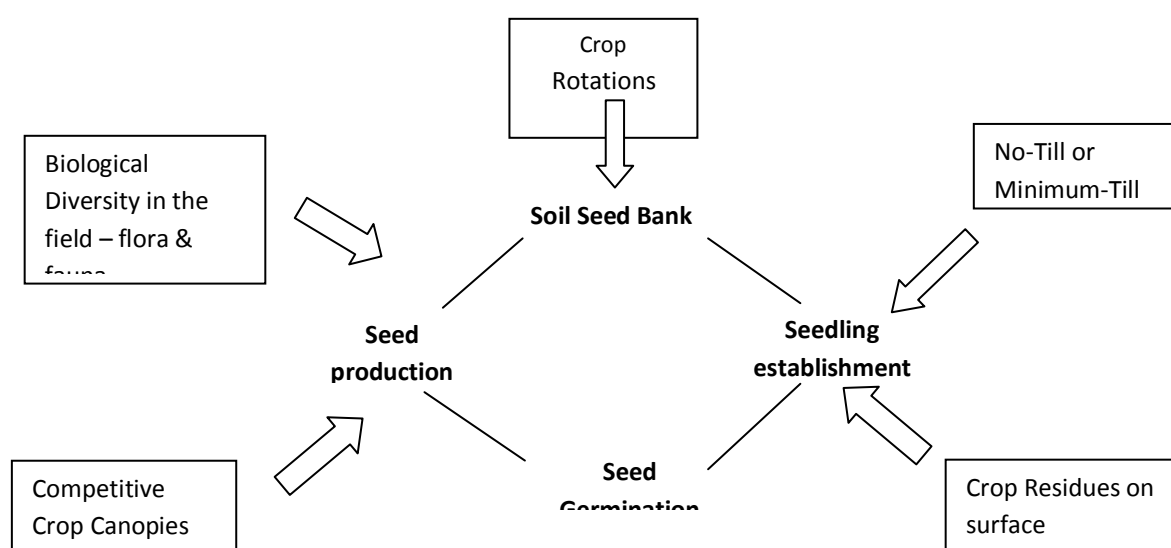


Figure 1. Components of an ecologically-based Weed Management System; each tactic can be manipulated to reduce weed population dynamics and disrupt weed abundance in fields

- Focus Weed Research on ecological systems - New weed control practices should be attractive to small farmers, and should serve to reduce weed stands and increase crop yields. At the same time, the practices should not have negative impacts on the ecological balance and ecosystem function; nor should they induce problems to human health. The focus of weed research should switch from simple trials and comparative studies of herbicide effectiveness to ecological studies. Systems analysis of weed species, weed communities and agro-ecosystems, should find alternatives to reduce the efforts farmers put towards weed control, at all scales of farming. Weed research should be conducted in both farmers' field areas as well as at research stations. Farmers can become effective partners in these efforts, so it is advisable to train them in methods for scouting fields and identifying weed species.
- Capacity Building: Focus on formal Tertiary-Level Weed Science Education - To develop truly integrated weed management systems, it is necessary to incorporate studies of weed biology, weed ecology and ecological principles that support sustainable management systems. To achieve this, it is necessary to strengthen the

training in plant identification, physiology and ecology of weeds, and their interactions with crops. This requires a closer interaction between 'pure science' and 'agricultural science' disciplines. Scientists from the two disciplines can make a vast difference if they work cooperatively to solve invasive species management problems.

DISCUSSION AND CONCLUSION

There is global concern that "...*biological diversity is being significantly reduced by human activities...*" (MFE, 1999). The International 'Convention on Biological Diversity' 1992 recommended that countries take action to: "...*prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species...*" This is because weeds threaten Biodiversity, one of the pillars on which nations can build sustainable futures. However, humans are culpable of spreading weeds, and 'human activities' are directly or indirectly linked to the most serious of weed problems. This 'human-dimension' cannot be ignored or downplayed, and needs to be addressed appropriately, by any country, planning to tackle weeds on a national scale.

Despite developing a national framework, Sri Lanka, as a developing country, is still some distance away from having the human resources and the capacity to implement holistic weed management programs to protect all our natural resources. However, steps in the right direction are being taken, and we believe that in the next five years or so, there would be significant increases in national competencies in Integrated Weed Management, which would be beneficial to the region as a whole. When implemented, Sri Lanka's NWS will be the keystone national framework to reduce the economic, environmental and social impacts of weeds in Sri Lanka, across landscapes. Ideally, it will promote a culture of rational discourse and not malign plants, just because some are problematic in some situations.

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INTEGRATED WEED MANAGEMENT IN FLOWER CROPS INVOLVING GOAT GRAZING AND POLYETHYLENE MULCHING

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ABSTRACT

On-farm participatory field experiments in southern India compared the efficiency of goat grazing and polyethylene mulching in tuberose (*Polianthes tuberosa* L.) grown by the development partners of Pudhupalayam cluster comprising three villages viz., Padiagraharam, Devanenthal and Periyeri in the National Agricultural Innovation Project implemented by Annamalai University. Results of two year experiments indicated that goat grazing reduced the infestation of *Cyperus rotundus* by 72 per cent and *Trianthema portulacastrum* by 23% .. Mulching with polyethylene film of 100µ thickness for 40 days from May reduced the *Trianthema portulacastrum* by 84%, whereas the same proved ineffective on *Cyperus rotundus*. Integration of goat grazing at 15 days interval in the off-season from March to May with polyethylene mulching during May before growing tuberose in middle of June performed better than traditional hand weeding and herbicide treatments in terms of weed control and crop performance.

Keywords: integrated weed management, flower, *Polianthes tuberosa* L.

INTRODUCTION

As the commercial floriculture has increased importance in the recent times the flower growing in India is carried out as a part of regular agricultural system on small holdings. Tuberose (*Polianthes tuberosa* cv. 'Single') is one of the popular and commercially important bulbous flower crops cultivated in an area of more than 3000 ha for loose flowers in Tamilnadu state of Indian subcontinent. One of the main constraints in the commercial cultivation of flower crops is weeds. Weeds cause irreparable damage to crops by competing for water, nutrients, light and space, besides acting as alternate hosts to a number of pathogens and insect pests (Shalini and Patil 2006). Manual weeding is time consuming and costly as the labour is scarce. Hence, it is imperative to employ alternate methods of weed control in flower cultivation irrespective of size of the holdings.

Chemical weed control is one of the alternate methods of weed control in flower crops. However, if herbicide accumulates in the soil, repeated applications can result in a reduction of crop growth (Sorkin 1981) and the development of herbicide resistance (Vanden and Balderston 1974). Soil persistence of herbicides can seriously affect the next crop in the rotation (Zaragoza 2003). Therefore, integrated approaches involving animal grazing and mulching are considered as feasible options for sustainable weed control in farming. Efficiency of Polyethelene mulching in weed control was better than that of Pendimethalin at a rate of 1 kg a.i./ha in flower crops (Shalini and Patil 2006). Grazing by

goats can control many weeds as they are capable of browsing on and controlling many spiny and noxious weeds (Ianpopay and Rogerfield 1996).

Under rainfed upland conditions, integrating goat rearing, especially for grazing during the off-season complimented weed control by 45% and addition of goat manure to fields grazed upon by goats contributed for 31% weed control during the cropping season (Kathiresan 2009). Further to improve weed management strategies participatory approach is required involving participation of growers and scientists (Geoff, 1996). In the participatory field trials conducted in uplands weed biomass was reduced from 23-29% because of grazing in the off season (Kathiresan 2010). In this study, on-farm participatory field experiments compared the efficiency of goat grazing and polyethylene mulching in tuberose (*P. tuberosa* L.) crops.

MATERIAL AND METHODS

The experiments were conducted during 2008-2009 and 2009-2010 in *P. tuberosa* L. grown by the development partners of Pudhupalayam cluster comprising three villages viz., Padiagraharam, Devanenthal and Periyeri in the National Agricultural Innovation Project implemented by Annamalai University. The experiment comprised of 10 treatments. Each treatment was adopted in an area of 1000 m² at selected small farmer's holdings and replicated in five fields. Fifty farmers participated in this experiment by adopting one treatment each. The treatments were compared in randomized block design. The tuberose bulbs were planted in a spacing of 20 x 30 cm during July 2008 and maintained up to March 2010. Then bulbs were harvested and preserved for next year planting during July 2010. Farm yard manure was applied at a rate of 30 t/ha. A fertilizer rate of 100:50:50 kg NPK/ha was applied. Standard cultural practices were followed.

Treatments

- T₁ -Off season grazing + Un-weeded control
- T₂ -Off season grazing + Hand weeding
- T₃ -Off season grazing + Pendimethalin @1 kg a.i. /ha
- T₄ -Off season mulching + Un-weeded control
- T₅ -Off season mulching + Hand weeding
- T₆ -Off season mulching + Pendimethalin @1 kg a.i. /ha
- T₇ -Off season fallow + Un-weeded control
- T₈ -Off season fallow + Hand weeding
- T₉ -Off season fallow + Pendimethalin @1 kg a.i. /ha
- T₁₀ -Off season grazing + polyethylene mulching

For the grazing treatments two goats issued through the NAIP project grazed daily during the off-season from March to May in the early morning hours and in late evenings. Mulching with polyethylene film of 100µ thickness was done for 40 days from May. To integrate these two practices goat grazing at 15 days interval in the off-season from March to May with polyethylene mulching during May before growing tuberose in middle of June was adopted. For rest of the treatments the land was maintained fallow during the off season. The super imposing treatments of un-weeded control, hand weeding thrice at 25th,

50th and 75th days after planting and application of Pendimethalin at 1 kg a.i./ha (Stomp Extra[®] 30% EC) were adopted as per treatment schedule. Pendimethalin at 1 kg a.i./ha was applied as pre-emergence herbicide within 72 h of planting. The observations of weed numbers, weed biomass and weed control index were recorded from four quadrates of 0.25 m² area at 45th and 90th days after planting and the data for the later date only is presented. Weed control index was calculated using the formula suggested by Thakur (1994). The data on dry matter production, loose flower and bulb yield were also recorded. The data on weed count were transformed by using the formula $\sqrt{x+0.5}$. All the data were analysed using the analysis of variance to draw the standard error and the critical difference was worked out at 0.5% probability as suggested by Panse and Sukhatme (1978).

$$\text{Weed control index} = \frac{a - b}{a} \times 100$$

Where, a – Weed biomass of un-weeded plot
b – Weed biomass of the treated plot

RESULTS

The weed flora observed in the experimental fields at all the three villages comprised the following species: *Cyperus rotundus*, *Trianthema portulacastrum*, *Dactyloctenium aegyptium*, *Cynodon dactylon*, *Corchorus olitorius* Linn., *Sida spp.*, *Amarantus spp.*, *Phyllanthus niruri*, *P.madraspatensis*, and *Cleome viscosa* Linn. Among these species *C.rotundus*, *T. portulacastrum* and *D. aegyptium* were the predominant weeds due to their frequent occurrence in all seasons and at all experimental fields (Table 1). Occurrence of other species was sporadic and at low densities. Among off season weed management practices grazing and polyethylene film mulching significantly reduced the population of weeds when compared to fields kept fallow during off season.

Table 1. Effect of integrated weed management options involving goat grazing and polyethylene mulching on the densities of three weeds species in *P. tuberosa* L.

Treatment Details	<i>Cyperus rotundus</i> / m ²		<i>Trianthema portulacastrum</i> /m ²		<i>Dactyloctenium aegyptium</i> / m ²	
	2008- 2009	2009- 2010	2008- 2009	2009- 2010	2008- 2009	2009- 2010
Off season grazing + Un-weeded control	6.4 (40.5)	6.2 (37.6)	4.5 (19.9)	2.1 (4.1)	3.1 (9.1)	2.9 (8.2)
Off season grazing + Hand weeding	3.8 (13.5)	3.3 (10.2)	2.1 (3.9)	1.6 (2.0)	1.8 (2.6)	1.6 (2.1)
Off season grazing + Pendimethalin @ 1kg a.i./ha	4.0 (15.6)	3.6 (12.6)	2.2 (4.4)	2.0 (3.6)	1.8 (2.8)	1.7 (2.4)
Off season mulching + Un-weeded control	9.4 (88.5)	8.7 (75.3)	2.2 (4.3)	2.1 (4.1)	3.8 (14.3)	3.6 (12.4)
Off season mulching + Hand weeding	4.9 (23.54)	4.2 (17.3)	1.6 (2.3)	1.5 (1.9)	2.4 (4.9)	2.0 (3.5)
Off season mulching+Pendimethalin @ 1 kg a.i./ha	5.4 (28.5)	4.5 (20.2)	1.8 (2.7)	1.6 (2.1)	2.4 (5.4)	2.3 (4.6)

Off season fallow + Un-weeded control	11.3 (126.4)	11.6 (134.5)	5.1 (24.7)	5.2 (26.1)	5.6 (30.4)	5.7 (32.2)
Off season fallow + Hand weeding	6.4 (40.2)	6.7 (44.6)	2.9 (7.8)	3.1 (8.9)	3.7 (13.4)	3.6 (12.7)
Off season fallow + Pendimethalin @1 kg a.i/ha	7.9 (61.5)	8.0 (64.2)	2.9 (8.4)	3.1 (9.4)	4.3 (18.4)	4.5 (19.5)
Off season grazing + polyethylene mulching	3.34 (10.9)	2.6 (6.4)	1.5 (1.8)	1.4 (1.4)	1.5 (1.9)	1.5 (1.6)
Standard Error difference.	0.2	0.2	0.2	0.2	0.1	0.1
Critical Difference(P=0.05)	0.4	0.5	0.4	0.5	0.2	0.3

(Figures in parenthesis are original values) Off season mulching with polyethylene film

The densities *C. rotundus* and *D. aegypticum* are concerned off season grazing significantly reduced the weed count when compared to polyethylene mulching, however, polyethylene mulching performed better in case of *T. portulacastrum*. When off season treatments are integrated with hand weeding and Pendimethalin at 1 kg a.i./ha weed densities of all the three weed species were significantly reduced and integration of hand weeding was better than the application of pendimethalin at 1 kg a.i./ha when combined with off season treatments like grazing and mulching. Integrating the off season weed control measures i.e., off season grazing + polyethylene mulching evinced the maximum reduction in weed count of major weeds in both the cropping years.

The total weed numbers showed similar results (Table 2.). The least total weed count of 17.5/m during 2008-2009 and 13.5/m during 2009-2010 were observed due to off season grazing + polyethylene mulching. Among the integrations between off season treatments and conventional weed control practices, off season grazing + hand weeding was better in reducing total weed numbers followed by off season grazing + pendimethalin @1kg a.i/ha. In general weed bio mass and weed control index (WCI) were significantly reduced due to grazing and mulching treatments during off season when compared to fallow fields. When results on weed characters of two years are compared the weed bio mass was reduced in off season treated fields in subsequent years and in fallow lands the weed bio mass was enhanced. In subsequent years WCI was increased in off season treated fields and reduced in fallow lands.

Table 2. Effect of integrated weed management options involving goat grazing and polyethylene mulching on weed numbers, biomass and control index in *P. tuberosa* L.

Treatment Details	Total weed numbers/m ²		weed biomass g/m ²		weed control index	
	2008-2009	2009-2010	2008-2009	2009-2010	2008-2009	2009-2010
Off season grazing + Un-weeded control	8.7 (75.4)	8.5 (72.1)	67.7	64.5	62.5	65.2
Off season grazing + Hand weeding	4.9 (24.2)	4.5 (20.1)	22.1	17.5	87.8	90.6
Off season grazing + Pendimethalin @1kg a.i/ha	5.2 (26.9)	4.8 (22.4)	24.1	19.5	86.6	89.5
Off season mulching + Un-weeded control	10.5 (110.4)	10.2 (104.4)	80.1	78.4	55.9	57.7
Off season mulching + Hand weeding	5.8	5.5	30.2	28.6	83.2	84.6

	(33.3)	(30.3)				
Off season mulching+Pendimethalin @ 1 kg a.i/ha	6.4 (40.1)	6.1 (36.5)	36.5	33.6	79.8	81.9
Off season fallow + Un-weeded control	14.5 (210.4)	14.8 (219.3)	180.6	185.6	-	-
Off season fallow + Hand weeding	8.1 (64.3)	8.2 (67.5)	57.6	60.2	68.1	67.5
Off season fallow + Pendimethalin @1 kg a.i/ha	9.5 (90.3)	9.9 (98.)	80.1	84.1	55.6	54.6
Off season grazing + polyethylene mulching	4.2 (17.5)	3.7 (13.5)	17.64	12.5	90.2	93.2
Standard Error difference.	0.2	0.3	1.4	1.6	1.0	0.8
Critical Difference(P=0.05)	0.5	0.6	3.1	3.6	2.0	1.8

(Figures in parenthesis are original values) Off season mulching with polyethylene film

When off season treatments are integrated with hand weeding and herbicide application, off season grazing + hand weeding performed better in reducing biomass and increasing WCI. This treatment was on par with season grazing + pendimethalin at 1kg a.i./ ha and superior to season mulching + hand weeding. The least biomass (17.6 g/m² and 12.5 g/m² during first and second year respectively) and the highest WCI (90.2 and 12.5 during first and second year respectively) were observed in integrating off season grazing + polyethylene mulching as integration options for weed control.

Growth and yield parameters of *P. tuberosa* were significantly influenced by the integrated weed management practices. Off season treatments enhanced the dry matter production, bulb yield and flower yield when compared to off season fallow. Among the off season treatments grazing excelled the polyethylene film mulching. When off season treatments are integrated with conventional practices these parameters were enhanced to the further. Integration of off season grazing + hand weeding performed better in terms of all yield parameters when compared to off season grazing + pendimethalin at 1kg a.i/ha and off season mulching + hand weeding. The highest dry matter production (391g/m² and 394g/m² during first and second year respectively), bulb yield (23.7 t/ha and 22.4 t/ha during first and second year respectively) and flower yield (12.8 t/ha and 13.8 t/ha during first and second year respectively) were recorded in Integration of goat grazing at 15 days interval in the off-season from March to May with polyethylene mulching during May before raising tuberoses in middle of June.

Table 3. Effect of integrated weed management options involving goat grazing and polyethylene mulching on dry matter production and yield of *P. tuberosa* L.

Treatment Details	Dry matter production g/m ²		Bulb yield (t/ ha)		Flower yield (t/ ha)	
	2008- 2009	2009- 2010	2008- 2009	2009- 2010	2008- 2009	2009- 2010
Off season grazing + Un-weeded control	267	270	16.2	16.1	9.5	9.7
Off season grazing + Hand weeding	374	376	21.0	20.8	11.5	11.9
Off season grazing + Pendimethalin @ 1kg a.i/ha	351	352	19.5	18.7	10.6	10.9
Off season mulching + Un-weeded control	286	287	15.1	15.1	8.7	8.7

Off season mulching + Hand weeding	351	353	19.8	18.9	10.5	10.7
Off season mulching+Pendimethalin @ 1 kg a.i/ha	315	317	17.5	17.1	9.1	9.3
Off season fallow + Un-weeded control	214	208	13.9	14.5	7.9	8.1
Off season fallow + Hand weeding	319	320	17.9	17.2	9.1	9.5
Off season fallow + Pendimethalin @1 kg a.i/ha	291	294	15.5	15.7	8.8	9.1
Off season grazing + polyethylene mulching	391	394	23.7	22.5	12.8	13.8
S.Ed.	3.0	4	0.3	0.3	0.2	0.2
CD(P=0.05)	7.0	8	0.6	0.6	0.5	0.4

Off season mulching with polyethylene film

DISCUSSION

Among the off-season treatments compared, goat grazing proved its superiority compared to polyethylene mulching and fallowing in suppressing the weed growth during the succeeding crop of tuberose. Repeated goat grazing of the shoot growth of *C.rotundus* likely depleted the food reserves of the underground tubers resulted in lesser infestation of the weed in cropping season. Though polyethylene mulching could kill the weed seeds of *T. portulacastrum*, *D. aegypticum* and other annual weeds, the heat flux accumulated in the soil strata is inadequate to kill or deplete the underground propagule as observed by Kumar *et al.*(1993), Miles *et al.* (2002), Webster (2003) and Johnson III *et al.* (2007). As *C. rotundus* was the dominant species in the floristic composition of weeds, goat grazing was effective. However, the integration of goat grazing and polyethylene mulching reduced weeds generally across a range of species during the cropping period, compared to the traditional weed control practice of hand weeding or herbicide application. It is likely that the soil weed seed bank is exhausted by the combined effect of grazing and polyethylene mulch that tackles the annual as well as perennial weeds. All the farmers participated in implementation of the research are convinced of the use of goats for sustainably managing weeds and are impressed with the management of perennial weeds particularly *C. rotundus* with this integrated management.

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POST-ENTRY RISK ASSESSMENT OF INVASIVE ALIEN FLORA IN SRI LANKA - PRESENT STATUS, GAP ANALYSIS, AND THE MOST TROUBLESOME ALIEN INVADERS

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ABSTRACT

The paper reviews the existing situation on risk assessment processes carried out globally to regulate introduction, early detections and management strategies for IAS globally and in the Sri Lankan context, and provides results of important activities carried out to develop a post-entry risk assessment protocol for invasive alien flora. The national lists of invasive alien species developed previously in Sri Lanka have not gone through a rigorous risk assessment process. The present study has led to the development of a post-entry risk assessment protocol for invasive alien flora in Sri Lanka through a participatory approach and based on a scientifically sound procedure. This has resulted in identification of 12 species of invasive alien flora in each category of National List (significantly threatening the environment) and Priority List (require urgent attention to tackle the problem) in Sri Lanka. The results are expected to foster an enabling policy, institutional and planning environment towards effective and informed-decision making, and to coordinate actions in tackling this major environmental issue. Steps need to be taken to generate and share knowledge about the rationale, need and specific techniques and best practices to tackle IAS in Sri Lanka through the use of valid risk assessment protocols.

INTRODUCTION

The significance of invasive alien species (IAS) as a global problem is widely recognized and the adverse effects of these species have been well described, including the situation in Sri Lanka (Marambe *et al.*, 2001; Marambe *et al.*, 2003; Marambe, 2008). The identification and characterization of the adverse effects associated with IAS is crucial, not only to prevent their introduction to new areas but also to prioritize management and control measures for already established IAS.

Risk assessment is a tool that can be used to support the exclusion of potential IAS from being introduced, as well as to assess the potential impact of those invasive species that have already become established. Risk assessment is one of the three components of risk analysis (Anderson *et al.*, 2004), and should be closely linked to the other two components; risk management and risk communication. Risk assessment protocols related to impacts of IAS on native biota and natural landscapes have been developed and

implemented by various international bodies such as the International Plant Protection Convention (www.ippc.int/id/13399), World Organization for Animal Health (www.oie.int) and Pacific Island Ecosystem at Risk (www.hear.org/pier/), and countries such as Australia (Downey, 2006), New Zealand (Williams *et al.*, 2000), Hawaii (Denslow *et al.*, 2000), Japan (Yoshioka, 2005), Canada (Anonymous, 2003), USA (www.plant_materials.nrcs.usda.gov/technical/invasive.html) etc., for regulation of alien species introductions, early detection and implementation of control/eradication measures. Risk analysis for issues related to entry, establishment and spread of IAS have been addressed through species- or habitat-based approaches in theoretical ecological studies such as pathway analysis, population viability analysis and by use of various mathematical models. Risk assessments, especially on weeds, are not only being conducted at country borders to forecast the consequences of a particular species introduction but also at post border level for those already present in the country or region, or which are likely future incursions so as to prioritize species for control and management processes (FAO, 2006).

Sri Lankan Context

At present, there are some legislative provisions directly addressing the prevention of introduction and control of spread of IAS in Sri Lanka (Marambe and Gunawardena, 2010). Pest risk assessment conducted at the National Plant Quarantine Services (NPQS) at Katunayake and the Seed Certification Center, Gannoruwa qualitatively investigates the likelihood of the entry, establishment and spread of aquatic or terrestrial pests including invasive plants, pathogens, nematodes and agricultural insects via imports of plants and planting material for non consumption and consumption purposes. These risk assessments comply with the International **Standards** for Phytosanitary Measures (ISPM standards) and often other institutions are consulted especially with regard to the prevention of introduction of invasive plant species to the country. At present, a more comprehensive protocol for screening of invasive species at the port of entry is being drafted by the NPQS of Sri Lanka.

Currently, risk analysis for already established IAS in Sri Lanka has been poorly addressed and has not been incorporated to IAS management strategies of the country. However, two preliminary post-entry risk assessment protocols for invasive alien flora have been developed, aimed at prioritization of IAS for management strategies: a protocol for prioritization of established invasive alien flora prepared by Hafiz *et al.* (2006), and one developed by Weerakoon (2007) for the Protected Area (PA) Management and Wildlife Conservation Project of the Department of Wildlife Conservation, Sri Lanka. Implementation of risk assessments for the control of introduction, establishment and spread of IAS in Sri Lanka is currently neglected primarily due to the lack of an acceptable assessment scheme to characterize risks associated with IAS in Sri Lanka. Gaps in knowledge, access and sharing of data and lapses in policies, responsibilities and applications are also attributed to this situation.

Gap Analysis

As in other risk assessment schemes developed for IAS in various countries, the risk assessment protocol for IAS in Sri Lanka could also address the economical, ecological

and social impacts, invasiveness, distribution, spread and management options of the known or potential invasive species. The principal state organization mandated for biodiversity conservation (the national focal point of CBD), the Biodiversity Secretariat of the Ministry of Environment, Sri Lanka, has a major role to fill gaps in risk assessments to manage the entry, establishment, spread and control measures for IAS in Sri Lanka.

Knowledge gaps

Risk analysis of IAS is totally based on existing scientific knowledge and risk assessment protocols assume that information on the target organism is readily available. Limitations in existing knowledge, especially on ecological aspects of IAS, often hamper identification and characterization of the 'invasiveness' of a species. Many species of invasive aliens at the early lag phase of invasions or that may be future/potential invaders, have still not been subjected to adequate scientific studies. Taxonomic relationships, invasiveness of relatives and history of invasiveness elsewhere are integral components of prediction of invasiveness. High ecological and taxonomical diversity of noxious species may result in difficulties in such investigations. The limited available information on growth rates, length of juvenile period, habitat requirements, dispersal patterns, behavioral studies etc, also constrains the identification of invasiveness. Further, knowledge of economical aspects related to IAS has been poorly explored and integration of economics with management and control programmes of IAS has occurred only for a few well known species. These limitations are a gap in risk assessment and risk analysis processes.

Data gaps

Information needed for assessment of invasiveness of a species and its impacts are often obtained through published and unpublished literature, scattered throughout different institutions, including personal communications. The lack of a repository of relevant information in an accessible and updated form, and of an adequate information management system for IAS, often causes problems in completing risk assessments.

Limited institutional facilities for quick retrieval of IAS information also create difficulties and has become a major problem especially for stakeholders conducting risk assessments at national borders. Limited data can lead to arbitrary decisions even on sanitary and phytosanitary measures, increasing the risks of noxious invaders being intentionally imported. Inefficient data sharing mechanisms among relevant stakeholders is another major reason for the gaps in data related to IAS.

Gaps in policies and applications/responsibilities

Lack of government policy on IAS is another constraint resulting in weak implementation of risk assessments for IAS in Sri Lanka. Poor understanding and awareness of impacts of IAS among politicians, policy makers, administrators, and lack of collaboration among relevant stakeholders also limits applications of risk assessments at border and post border levels. Structural and management deficiencies in organizations/institutions, usually due to shortages in financial and/or human resources, can disable the institutional capability to conduct effective risk assessments for IAS.

In certain situations deficiencies in international regulatory frameworks relating to international standards can contribute to a lack of policies for application of a risk analysis process. The International Convention for the Control and Management of Ships' Ballast

Water and Sediments (2004) highlights management options to control the spread of marine organisms and pathogens during ballast water exchanges. However, it does not specifically emphasize invasive alien planktonic organisms or the need for a risk analysis of ballast water exchange processes. Deficiencies in international regulatory frameworks are also present in IPPC as there is no direct provision to address IAS, which are not considered as pests. There is also a concern about the exotic pathogens and parasites that could pose risks to wildlife but that are not listed by the OIE (www.oie.int).

Post-entry Risk Assessment for Invasive Alien Flora in Sri Lanka

The implementation of risk assessment protocols to prevent further introduction of IAS to Sri Lanka was first highlighted a decade ago, at the First National Workshop on IAS (Marambe, 1999). Later, the urgent need to establish a standardize surveillance and a threat assessment of IAS in protected areas was emphasized in the proposed policy on control of invasive species in protected areas of Sri Lanka (Weerakoon, 2007). The addendum to the Biodiversity Conservation (MENR, 2007) also recommended the preparation of risk assessment protocols for prioritization of established invasive species in Sri Lanka.

Although risks associated with alien plant introductions are considered at the points of entry by the quarantine authorities, IAS have not been specifically addressed in standard regulations for species introductions. Prevention is considered the most economical and effective management strategy against alien invaders (Wittenberg and Cock, 2001). Hence, a better prediction of whether or not a species is likely to be invasive should be conducted at the national borders, to reduce subjectivity in judgments, and minimize both over and under-estimates of risks associated with IAS.

Risk assessments for IAS are used to select species that warrant immediate control as most aggressive species are identified by the screening process. Current lists of invasive plants and animals in Sri Lanka (Bambaradeniya, 1999, 2000, 2002; Marambe *et al.*, 2001, 2003) have not been developed through risk evaluation, and there is a need for a (qualitative and/or quantitative) assessment scheme to measure relative risks of IAS in Sri Lanka. An attempt at this was made in 2009-2010, through a broad stakeholder consultation and with technical expertise provided by the United Nations Development Organization (UNDP) – Sri Lanka and the Global Environmental Facility (GEF). The protocol presented has been accepted by the Ministry of Environment of Sri Lanka and will be used for prioritization of the invasive alien flora in order to develop management strategies and decide on financial allocations in the future.

The risk assessment protocol was designed to include four main themes, each divided into segments covering important areas (Table 1). Each sub-section was addressed in detail with a set of questions with 3-5 potential responses per question, scored from least likely (0) to most likely (4). The questions were designed to ensure minimum occurrence of the response 'unknown'.

Table 1. Major themes and sub-themes in the post-entry risk assessment protocol for invasive alien flora

Theme	Sub sections	No.of questions
(1) Potential of Ecological and Socio-Economic Impacts	Potential impacts on ecosystem processes	1
	Potential impacts on community structure	1
	Potential impacts on community composition	1
	Potential impacts on other trophic levels	1
	Potential impacts on genetic integrity	1
	Social nuisance and potential injuries to human health/injury risks	1
	Potential impacts on landscape diversity and aesthetic aspects	1
	Any beneficial use	1
	If used commercially, could it lead to harmful impacts	1
	Potential detrimental impacts on agricultural/forestry/fishery productivity	1
	Any socio-political, religious, or ethical considerations	1
(2) Invasive potential	Propagation	5
	Establishment and/or spread due to disturbances	2
	Competitive ability	5
(3) Distribution	Current global distribution	1
	Known level of impact in natural areas at global scale	1
	The species domesticated and/ or reported as a weed elsewhere	1
	Distributions in Asia as an exotic species	1
	Potential distribution in Sri Lanka	1
(4) Management options	Chances of re-introduction in future	1
	Likelihood of entry through unintentional introductions	1
	Possibility of detection if escaped from manageable areas	1
	Level of effort required for management if escaped	1

The scoring was done by 63 people actively involved in IAS activities in Sri Lanka, including academics, policy makers, scientists, researchers, and extension officers in the state, private sector and international and national non-governmental organizations. The input of 15 practitioners were also used in determining the final scores, and selected questions from themes 1-3 were given double weight. The invasive alien flora from previous lists prepared without a risk assessment protocol (Bambaradeniya, 2002; Marambe *et al.*, 2003) were evaluated individually according to the listed criteria, and the total scores for each species for each section calculated. The maximum possible score for each section was determined and the scores obtained expressed as a percentage of this.

Preparation of National and Priority lists for invasive alien flora in Sri Lanka

Development of the National List was based on impact severity of the species, using only the rating from themes 1-3 (impacts, invasive potential and distribution), with the overall severity score calculated as a weighted percentage. Species with a score >70% were placed in the National List of Invasive Alien Flora, those that significantly affect environmental values of the country. Species with a score 40-70% were considered Potential Invasive Species. The Priority List of invasive alien flora, where management strategies are urgently needed, was based on the severity score obtained above and the total score obtained for section 4 (management option), expressed as a percentage of the maximum. Species with a score > 70% were considered priority species that requires immediate action. The results were validated by a broad stakeholder group of administrators and scientists involved in IAS control in Sri Lanka. The resulting National list and the Priority invasive alien flora list for Sri Lanka, totalling 12 species in each category (dirty dozen), are given in Table 2.

Table 2. National and Priority lists of Invasive Alien Flora of Sri Lanka from post-entry risk assessment

Species	National List (scores for themes 1, 2 and 3)	Priority List (scores incl. theme 4)
Priority Invasive Alien Flora		
<i>Panicum maximum</i>	1	1
<i>Pennisetum polystachion</i>	2	2
<i>Eichhornia crassipes</i>	3	7
<i>Salvinia molesta</i>	4	5
<i>Lantana camara</i>	5	3
<i>Mimosa pigra</i>	6	8
<i>Prosopis juliflora</i>	7	6
<i>Opuntia dillenii</i>	8	4
<i>Clidemia hirta</i>	9	9
<i>Dillenia suffraticosa</i>	10	12
<i>Austroeupatorium inulifolium</i>	11	10
<i>Ageratina riparia</i>	12	11
Potential Invasive Alien Flora		
<i>Mikania micrantha</i>	13	
<i>Clusia rosea</i>	14	
<i>Miconia calvenscens</i>	15	
<i>Sphagneticola trilobata</i>	16	
<i>Pennisetum clandestinum</i>	17	
<i>Parthenium hysterophorus</i>	18	
<i>Chromolaena odorata</i>	19	
<i>Alstonia macrophylla</i>	20	
<i>Cuscuta campestris</i>	21	
<i>Myroxylon balsamum</i>	22	
<i>Tithonia diversifolia</i>	23	
<i>Swietenia macrophylla</i>	24	
<i>Cestrum aurantiacum</i>	25	
<i>Aristea ecklonii</i>	26	
<i>Psidium littorale</i>	27	
<i>Ulex europaeus</i>	28	

The order of priority in the National List differed in some cases to that of the Priority List, however, as a group, the dirty dozen remained the same. Variation within the group is mainly due to the management strategies adopted at present and related experiences of the stakeholders in the consultative process who identified some species as more invasive than others based on unpublished information. The final lists have given due recognition to the views of stakeholder organizations actively involved in management of IAS in different ecosystems.

The absence of scientifically valid risk assessment protocols and properly constituted National and Priority Lists has been a hindrance to efforts to control IAS in Sri Lanka. *Ad hoc* national lists prepared in the past have hampered the efforts of IAS control, diverting interest away from the actual situation. The risk assessment protocol developed in this study followed a participatory approach for post-entry risk assessment to identify invasive alien flora in Sri Lanka, which will foster an enabling policy, institutional and planning environment towards effective and informed joint actions, at the same time taking steps to generate and share knowledge about the rationale, need and specific techniques and best

practices to tackle IAS in Sri Lanka. Similar efforts need to be extended to invasive alien fauna and for pre-border risk assessment of IAS in Sri Lanka to safeguard its unique environments from the detrimental impacts of IAS.

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BIO - EFFICACY EVALUATION OF HERBICIDE RESISTANT TRANSGENIC CORN HYBRIDS FOR CROP SAFETY AND PRODUCTIVITY (MON 89034 X NK603)

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ABSTRACT

Herbicide resistant corn plants confer tolerance to glyphosate by production of the glyphosate-tolerant CP4 5-enolpyruvylshikimate-3phosphate synthase (CP4 EPSPS) proteins. As an initiative on transgenic corn in India, transgenic stacked corn hybrids evolved by Monsanto India Ltd., NK603 is the glyphosate tolerant technology for the effective weed management system. The plant becomes tolerant to the herbicide while all other weed flora suppressed after application of glyphosate. The transgenic corn hybrids were evaluated during *kharif* 2009 and *rabi* 2009-10 at experimental site of Tamil Nadu Agricultural University, Coimbatore, India. Glyphosate was applied as early POE application at 900, 1800 and 3600 g a.e/ha in Hishell and 900 M Gold transgenic corn hybrids compared with non-transgenic counterparts with PE atrazine at 0.5 kg/ha + HW on 40 DAS and with and without insect management. Early POE application of glyphosate at 900, 1800 and 3600 g a.e/ha reduced weed density, dry weight and WCE in transgenic Hishell and 900 M Gold corn hybrids compared to non-transgenic corn hybrids with herbicidal treatment. Higher grain yield was recorded with POE application of glyphosate at 900, 1800 and 3600 g a.e/ha in Hishell and 900 M Gold transgenic corn hybrids. Average yield obtained in transgenic corn hybrid was 10 t/ha and conventional transgenic corn hybrid was 8 t/ha.

Key words: Herbicide resistant corn, weed density, dry weight, herbicide residue.

INTRODUCTION

Genetic engineering is one form of biotechnology that is used to enhance the agronomic characteristics of plants by inserting a gene or sequence of genes that express desirable traits. The most successful example has been glyphosate-resistant technology. The ability to manipulate the plant genome directly gave scientists new ways to create corn crop tolerant to glyphosate.

Transgenic stack hybrid corn (MON 89034 X NK603) was developed for preventing yield losses of corn crop due to pests and weeds and to improve productivity. The stacked corn crop having both insect protection and herbicide tolerant traits will provide protection to the crop from target pests and also provide tolerance to glyphosate herbicide. MON 89034 is second generation Bt corn technology effective against lepidopteron insect pests with a unique and innovative dual mode of action. NK 603 is the glyphosate tolerant technology for the effective weed management system. The plants become tolerant to the herbicide while weed flora are suppressed after application of herbicide. Hence, field experiments

were carried out to evaluate the bio-efficacy, phytotoxicity, residue and carryover of potassium salt of glyphosate formulation on transgenic stacked corn hybrids” with the following objectives:

- ❖ To evaluate weed control efficiency with K salt of glyphosate formulation in transgenic stacked corn hybrids along with other selective herbicides in conventional hybrids.
- ❖ To study the effect of different weed management practices on corn growth and development

MATERIAL AND METHODS

Field experiments were conducted at the Eastern Block Farm of Tamil Nadu Agricultural University, Coimbatore, India. The farm is situated in western agro-climatic zone of Tamil Nadu at 77°E 11°N latitude 426 m above mean sea level and the farm receives the normal total annual rainfall of 674.2 mm in 45.8 rainy days. The soil of the experimental field was sandy clayey loam in texture with low available nitrogen, medium available phosphorus and high available potassium.

Treatment details

- T₁ - Transgenic stacked Hishell with POE glyphosate at 900 g a.e./ha*
- T₂ - Transgenic stacked Hishell with POE glyphosate at 1800 g a.e./ha*
- T₃ - Transgenic stacked Hishell with POE glyphosate at 3600 g a.e./ha*
- T₄ - Transgenic stacked 900 M Gold with POE glyphosate at 900 g a.e./ha*
- T₅ - Transgenic stacked 900 M Gold with POE glyphosate at 1800 g a.e./ha*
- T₆ - Transgenic stacked 900 M Gold with POE glyphosate at 3600 g a.e./ha*
- T₇ - Non-transgenic Hishell with PE atrazine at 0.5 kg ha⁻¹ on 3 DAS *fb* HW 40 DAS and insect control
- T₈ - Non-transgenic Hishell with no weeding and no insect control
- T₉ - Non-transgenic Hishell with no weeding and only need based insect control
- T₁₀ - Non-transgenic 900 M Gold with PE atrazine at 0.5 kg ha⁻¹ on 3 DAS *fb* HW 40 DAS and insect control
- T₁₁ - Non-transgenic 900 M Gold no weeding and no insect control
- T₁₂ - Non-transgenic 900 M Gold with no weeding and only need based insect control
- T₁₃ - Proagro 4640 with PE atrazine at 0.5 kg ha⁻¹ on 3 DAS *fb* HW 40 DAS and insect control
- T₁₄ - Proagro 4640 no weeding and no insect control
- T₁₅ - CoHM 5 with PE atrazine at 0.5 kg ha⁻¹ on 3 DAS *fb* HW 40 DAS and insect control
- T₁₆ - CoHM 5 no weeding and no insect control

* *Glyphosate application at 2 - 4 leaf stage of weeds (approximately 20-25 DAS of maize)*

Experiment details

Treatments	: 16
Replications	: 3

Design	: RBD
Gross Plot (3.6m x 5.0 m)	: 18 m ²
Rows per plot	: 6
Row length	: 5 m
Spacing between rows	: 60 cm
Spacing between plants	: 25 cm
Spacing between replications	: 1.5 m
No. of rows of African tall maize	: 13 (all four sides of experimental plot)
Gross experimental area including African tall	: 2903 sq m (77.2 m x 37.6 m)
Water volume for herbicide spray	: 250 l/ha for spray of herbicide
Spray equipment	: Knapsack sprayer fitted with flat fan nozzle

Observations were made on predominant weed flora and weed density and dry weight, corn yield, herbicide residue in both the trial.

RESULTS AND DISCUSSION

Predominant weed flora of the experimental field

Weed flora of the experimental fields consisted of predominantly ten species of broad leaved weeds, five species of grassy weeds and a sedge weed. The predominant broad leaved weeds were *Trianthema portulacastrum*, *Cleome gynandra*, *Boerhavia diffusa*, *Digera arvensis* and *Cyanotis axillaris*. Among the grass weeds, *Cynodon dactylon* and *Dactyloctenium aegyptium* were dominant. *Cyperus rotundus* was the only sedge weed found in the experimental fields.

During *kharif* 2009 broad leaved weeds dominated the weed flora (89.6%) and it was followed by sedges (5.8%) and grasses (4.5%) at 20 DAS. At 40 DAS and 65 DAS also broad leaved weeds were more (83.9 and 83.7%) followed by grasses (8.4 and 8.7%) and sedges (7.6 and 7.4%). During *rabi*, 2009-10 at 20 DAS, broad leaved weeds were more (74.7%) compared to grasses (15.5%) and sedges (9.7%). At 40 DAS and 65 DAS also broad leaved weeds (70.2 and 70.5%) were dominated the grasses (16.9 and 15.7%) and sedge (12.8 and 13.7%).

Weed density and dry weight

During *kharif* 2009 season lower weed density was recorded under non-transgenic Hishell and Proagro with herbicidal treatment. These were comparable with non-transgenic 900 M Gold and CoHM 5 corn hybrids with same herbicidal treatment at 20 DAS. Whereas at 40 DAS and 60 DAS lower weed density was recorded under transgenic 900 M Gold with POE application of glyphosate at 3600 g a.e/ha. During *rabi* 2009-10 non-transgenic Hishell with herbicidal treatment was recorded lower weed density. This was comparable with other non-transgenic corn hybrids with same herbicidal treatment. At 40 DAS POE

application of glyphosate at 1800 and 3600 g a.e/ha under transgenic Hishell and 900 M Gold corn hybrids registered significantly lesser weed density (Table 1).

Table 1. Effect of different weed management practices on the total weed density in corn

Treatments	Total weed density (No/m ²)*					
	Kharif 2009			Rabi 2009-10		
	20 DAS	40 DAS	65 DAS	20 DAS	40 DAS	65 DAS
T ₁ - T. Hishell POE glyphosate @ 900 g a.e/ha	15.81 (248.0)	3.61 (11.0)	3.83 (12.7)	12.10 (144.3)	4.24 (16.0)	3.96 (13.7)
T ₂ - T. Hishell POE glyphosate @ 1800 g a.e/ha	15.45 (236.7)	2.65 (5.0)	3.32 (9.0)	12.65 (158.0)	3.16 (8.0)	2.52 (4.3)
T ₃ - T. Hishell POE glyphosate @ 3600 g a.e/ha	15.39 (235.0)	2.00 (2.0)	2.45 (4.0)	12.03 (142.7)	2.08 (2.3)	2.00 (2.0)
T ₄ - T. 900 M Gold POE glyphosate @ 900 g a.e/ha	15.70 (244.4)	3.79 (12.3)	4.47 (18.0)	11.82 (137.7)	4.40 (17.3)	3.87 (13.0)
T ₅ - T. 900 M Gold POE glyphosate @ 1800 g a.e/ha	14.38 (204.7)	1.73 (1.0)	3.31 (9.0)	12.83 (162.7)	3.00 (7.0)	2.71 (5.3)
T ₆ - T. 900 M Gold POE glyphosate @ 3600 g a.e/ha	14.10 (196.7)	1.73 (1.0)	2.38 (3.7)	12.46 (153.3)	2.16 (2.7)	1.73 (1.0)
T ₇ - Hishell PE atrazine @ 0.5 kg ha ⁻¹ + HW+ IC	7.26 (50.7)	8.02 (62.3)	5.03 (23.4)	5.17 (24.7)	7.30 (51.3)	5.10 (24.0)
T ₈ - Hishell No WC and no IC	16.14 (258.4)	13.04 (168.0)	12.42 (152.3)	12.96 (166.0)	11.39 (127.7)	10.94 (117.7)
T ₉ - Hishell No WC and only IC	15.88 (250.3)	13.24 (173.3)	12.27 (148.6)	12.29 (149.0)	10.65 (111.3)	10.38 (105.7)
T ₁₀ - 900 M Gold PE atrazine @ 0.5 kg a.i/ ha+ HW+ IC	7.02 (47.3)	7.66 (56.7)	5.10 (24.0)	5.39 (27.0)	7.28 (51.0)	5.26 (25.7)
T ₁₁ - 900 M Gold No WC and no IC	14.86 (219.0)	13.29 (174.7)	12.40 (151.7)	12.64 (157.7)	11.82 (137.7)	10.68 (112.0)
T ₁₂ - 900 M Gold No WC and only IC	15.39 (234.7)	12.73 (160.0)	11.90 (139.7)	11.83 (138.0)	11.15 (122.3)	10.25 (103.0)
T ₁₃ - Proagro PE atrazine @ 0.5 kg a.i/ ha + HW+ IC	6.93 (46.0)	8.02 (62.3)	4.87 (21.7)	5.94 (33.3)	7.12 (48.7)	5.10 (24.0)
T ₁₄ - Proagro 4640 No WC and no IC	14.99 (222.6)	13.06 (168.7)	12.14 (145.3)	12.38 (151.3)	11.56 (131.7)	10.46 (107.3)
T ₁₅ - CoHM 5 PE atrazine @ 0.5 kg a.i/ ha + HW+ IC	7.21 (50.0)	7.72 (57.7)	4.90 (22.0)	5.48 (28.0)	7.24 (50.4)	5.20 (25.0)
T ₁₆ - CoHM 5 No WC and no IC	16.27 (262.6)	12.82 (162.3)	12.41 (152.0)	12.92 (165.0)	11.72 (135.3)	10.98 (118.7)
CD (P=0.05)	2.82	2.12	2.14	2.18	1.58	1.41

Figures in parenthesis are original values; * Data are square root transformed values

PE application of atrazine at 0.5 ka/ha *fb* HW recorded lesser dry weight of total weeds under CoHM 5 maize hybrid and this was comparable with all other non-transgenic corn hybrids with same herbicidal treatment at 20 DAS. At 40 DAS and 65 DAS lesser total weed dry weight was recorded with POE application of glyphosate at 3600 g a.e/ha under transgenic 900 M Gold corn hybrid. This was found to be comparable with other herbicidal treatments applied under both the transgenic hybrids during *kharif* 2009 season. During *rabi* 2009-10 non-transgenic 900 M Gold corn hybrid with PE application of atrazine at 0.5

kg/ha *fb* HW recorded lesser weed dry weight at 20 DAS. Whereas at 40 DAS total weed dry weight was lower with POE application of glyphosate at 3600 g a.e/ha applied in transgenic 900 M Gold corn hybrid and this was comparable all other herbicidal treatments applied under transgenic corn hybrids (Table 2).

Table 2. Effect of different weed management practices on the total weed density in corn

Treatments	Total weed dry weight (g/m ²)*					
	<i>Kharif</i> 2009			<i>Rabi</i> 2009-10		
	20 DAS	40 DAS	65 DAS	20 DAS	40 DAS	65 DAS
T ₁ - T. Hishell POE glyphosate @ 900 g a.e/ha	6.91 (45.76)	2.10 (2.40)	2.67 (5.11)	6.47 (39.81)	3.01 (7.09)	3.13 (7.77)
T ₂ - T. Hishell POE glyphosate @ 1800 g a.e/ha	7.19 (49.64)	1.76 (1.10)	2.32 (3.37)	6.76 (43.71)	2.35 (3.51)	2.13 (2.52)
T ₃ - T. Hishell POE glyphosate @ 3600 g a.e/ha	6.67 (42.48)	1.62 (0.62)	1.90 (1.62)	6.91 (45.72)	1.79 (1.20)	1.79 (1.21)
T ₄ - T. 900 M Gold POE glyphosate @ 900 g a.e/ha	7.07 (47.98)	2.23 (2.98)	2.95 (6.68)	6.06 (34.67)	3.26 (8.66)	3.06 (7.34)
T ₅ - T. 900 M Gold POE glyphosate @ 1800 g a.e/ha	6.66 (42.35)	1.51 (0.29)	2.48 (4.17)	7.08 (48.10)	2.29 (3.25)	2.21 (2.89)
T ₆ - T. 900 M Gold POE glyphosate @ 3600 g a.e/ha	6.51 (40.36)	1.53 (0.33)	1.85 (1.41)	6.40 (38.97)	1.82 (1.32)	1.62 (0.64)
T ₇ - Hishell PE atrazine @ 0.5 kg ha ⁻¹ + HW+ IC	3.48 (10.11)	5.06 (23.61)	3.49 (10.21)	3.22 (8.38)	5.39 (27.06)	3.71 (11.74)
T ₈ - Hishell No WC and no IC	6.82 (44.54)	10.07 (99.43)	9.87 (95.49)	7.21 (50.04)	8.80 (75.43)	9.40 (86.32)
T ₉ - Hishell No WC and only IC	7.34 (51.85)	10.39 (106.00)	9.84 (94.87)	6.79 (44.06)	8.25 (65.99)	9.00 (79.07)
T ₁₀ - 900 M Gold PE atrazine @ 0.5 kg a.i/ ha+ HW+ IC	3.52 (10.39)	5.33 (26.45)	3.60 (10.93)	3.17 (8.07)	5.29 (26.01)	3.98 (13.81)
T ₁₁ - 900 M Gold No WC and no IC	6.82 (44.45)	9.69 (91.92)	9.94 (96.78)	7.06 (47.79)	9.43 (86.89)	9.36 (85.64)
T ₁₂ - 900 M Gold No WC and only IC	7.44 (53.34)	10.03 (98.63)	9.67 (91.43)	6.23 (36.82)	8.68 (73.30)	9.05 (79.98)
T ₁₃ - Proagro PE atrazine @ 0.5 kg a.i/ ha + HW+ IC	3.49 (10.20)	5.24 (25.45)	3.72 (11.87)	3.49 (10.21)	5.29 (26.00)	4.21 (15.70)
T ₁₄ - Proagro 4640 No WC and no IC	7.47 (53.76)	9.51 (88.42)	9.78 (93.72)	6.93 (45.99)	9.09 (80.58)	9.33 (85.12)
T ₁₅ - CoHM 5 PE atrazine @ 0.5 kg a.i/ ha + HW+ IC	3.22 (8.39)	5.35 (26.67)	3.63 (11.18)	3.27 (8.70)	5.34 (26.50)	4.01 (14.10)
T ₁₆ - CoHM 5 No WC and no IC	7.99 (61.77)	10.29 (103.95)	10.31 (104.23)	6.70 (42.95)	9.51 (88.50)	9.71 (92.32)
CD (P=0.05)	1.41	1.42	1.44	1.20	1.49	1.00

Figures in parenthesis are original values; * Data are square root transformed values

Corn grain yield

During *kharif* 2009 season POE application of glyphosate at 1800 g a.e/ha in transgenic 900 M Gold corn hybrid resulted in higher grain yield of 12.01 t/ha (Table 3). This was 36.64 % higher than the unweeded check of non-transgenic 900 M Gold maize hybrid. Whereas during *rabi* 2009-10 season POE application of glyphosate at 3600 g a.e/ha in transgenic Hishell corn hybrid resulted in higher grain yield of 10.12 t/ha. This was 37.15 %t higher than the unweeded check plots of non-transgenic Hishell maize hybrid.

Table 3. Effect of different weed management methods on yield of corn

Treatments	Grain yield (t/ha)	
	<i>Kharif</i> , 2009	<i>Rabi</i> , 2009-10
T ₁ - T. Hishell POE glyphosate @ 900 g a.e/ha	11.19	8.96
T ₂ - T. Hishell POE glyphosate @ 1800 g a.e/ha	11.64	9.86
T ₃ - T. Hishell POE glyphosate @ 3600 g a.e/ha	11.78	10.12
T ₄ - T. 900 M Gold POE glyphosate @ 900 g a.e/ha	11.30	9.33
T ₅ - T. 900 M Gold POE glyphosate @ 1800 g a.e/ha	12.01	10.00
T ₆ - T. 900 M Gold POE glyphosate @ 3600 g a.e/ha	11.68	9.92
T ₇ - Hishell PE atrazine @ 0.5 kg a.i/ha + HW+ IC	10.52	8.89
T ₈ - Hishell No WC and no IC	7.57	6.36
T ₉ - Hishell No WC and only IC	8.05	7.21
T ₁₀ - 900 M Gold PE atrazine @ 0.5 kg a.i/ha + HW+ IC	10.27	9.27
T ₁₁ - 900 M Gold No WC and no IC	7.61	7.19
T ₁₂ - 900 M Gold No WC and only IC	8.14	8.37
T ₁₃ - Proagro PE atrazine 0.5 @ kg a.i/ha + HW+ IC	8.00	6.95
T ₁₄ - Proagro 4640 No WC and no IC	5.98	5.62
T ₁₅ - CoHM 5 PE atrazine @ 0.5 kg a.i/ha + HW+ IC	8.04	7.15
T ₁₆ - CoHM 5 No WC and no IC	6.08	5.73
CD (P=0.05)	1.46	1.69

DISCUSSION

Among the various rates of glyphosate, glyphosate at 1800 g a.e/ha in transgenic 900 M Gold (1.0 No's/m²) and 3600 g a.e/ha in transgenic Hishell (2.3 No's/m²) recorded lesser total weed density during *kharif* and *rabi* seasons, respectively at 40 DAS. Glyphosate at 900 g a.e/ha gave lesser control when compared to higher doses, also it failed to control weeds after 60 DAS. Lower dose of glyphosate was not effective in controlling *Cyperus rotundus* and some broad leaved weeds like *Commelina benghalensis* and *Cyanotis axillaris*. Koger and Reddy (2005) also found that, glyphosate provides marginal or no control of weeds such as *Cynodon dactylon*, *Solanum carolinense* and tropical *Commelina benghalensis*. Similarly, some plants in a dormant stage are tolerant to glyphosate, but this is due to a limitation in glyphosate absorption or translocation. In non-transgenic maize hybrids, application of atrazine at 0.5 kg/ha was proved as effective pre-emergence weed control option in maize. Atrazine effectively controlled majority of broad leaved and grassy weeds at earlier stages of maize growth. Mundra *et al.*, (2003) reported that application of atrazine at 0.5 kg/ha as pre-emergence *fb* inter cultivation at 35 DAS in maize significantly reduced the total weed density and weed dry weight.

Glyphosate at 1800 g a.e/ha in transgenic 900 M Gold and 3600 g a.e/ha in transgenic Hishell (0.29 and 1.20) at 40 DAS during *kharif* 2009 and *rabi* 2009-10 seasons respectively. This might be due to total weed control as achieved by glyphosate. The findings are in accordance with Reddy and Boykin (2010) who reported that POE application of glyphosate, following PE herbicides, or three applications of POE glyphosate only without PE herbicides reduced total weed dry weight by at least 97 % when compared to without glyphosate applied plots. Total weed dry weight was effectively reduced in non-transgenic hybrids with PE application of atrazine at 0.5 kg/ha *fb* HW. Kumar and Thakur (2005) also found that application of atrazine in corn recorded least weed dry weight.

The highest grain yield of maize was obtained with POE application of glyphosate at 1800 g a.e /ha in transgenic 900 M Gold (12.01 t/ha) and glyphosate at 3600 g a.e/ha in transgenic Hishell (10.12 t/ha) during *kharif* and *rabi* seasons, respectively. This could be attributed to efficient control of weeds during the cropping period. Tharp *et al.* (1999) who

had earlier reported that maize yields of herbicide resistant hybrids were maximum with glyphosate at 0.84 kg a.e/ha of glyphosate when applied at fifth leaf stage of maize.

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ACETOLACTATE SYNTHASE (ALS) INHIBITOR RESISTANT *ECHINOCHLOA* SPECIES EVOLVED IN KOREA AND ITS RESISTANCE MECHANISM

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ABSTRACT

Echinochloa oryzicola and *E. crus-galli* collected from Gimje and Seosan, Korea were tested with ALS inhibitors including azimsulfuron, to confirm if they were resistant to ALS inhibitors. Gimje biotype of *E. oryzicola* showed strong resistance to azimsulfuron with R/S ratio of greater than 300 in the whole plant test. ALS enzyme assay demonstrated that ALS of Gimje biotype had 8.7 times greater tolerance to azimsulfuron than that of Suwon biotype, a reference susceptible biotype. Organophosphate insecticide fenitrothion was used to evaluate if enhanced metabolism by the cytochrome P-450 monooxygenase (P450s) is related to this resistance. The R/S ratio of GR₅₀ values in the whole plant test was reduced from 327.7 to 10.2 by adding fenitrothion, suggesting that P450s may be involved in ALS inhibitor resistance in Gimje biotype. These results indicate that the ALS inhibitor resistance in *E. oryzicola* is associated with both target site and metabolism.

Keyword: Acetolactate synthase; *Echinochloa oryzicola*; azimsulfuron; fenitrothion

INTRODUCTION

Herbicide-resistant weed species in paddy fields throughout Korea have been steadily reported since the first resistance to ALS inhibitors was indentified in *Monochoria korsakowii* in Seosan Hyundai Farm, Chungnam province in 1999 (Park *et al*, 1999; Kwon *et al*, 2000; Park *et al*, 2001; Kuk *et al*, 2002; Im *et al*, 2003; Kuk *et al*, 2003; Im *et al*, 2005; Park *et al*, 2006; Im *et al*, 2009; Kwon *et al*, 2009). Therefore, management of herbicide resistance has become an important issue in Korea, and understanding the resistance mechanisms of herbicide-resistant weed species in rice is necessary to find alternative herbicide with different modes of action and to develop a new herbicide for managing herbicide-resistant weeds.

The target site-based resistance has most commonly occurred around the world and can be relatively easily managed by rotating herbicides with different modes of action (Preston, 2004). However, enhanced herbicide metabolism is less known for herbicide resistance mechanism and can be involved in multiple resistances of chemically unrelated herbicides (Siminszky, 2006). Management for enhanced metabolism-based resistance may be more complex than the target site-based resistance. Three enzymes are associated with herbicide metabolism, such as aryl acylamidases, glutathione-S-transferases and cytochrome P-450 monooxygenases (P450s), of which P450s are involved in diverse patterns of herbicide resistance compared to aryl acylamidases and glutathione-S-transferases (Preston, 2004). Previous studies reported that P450s have been implicated

in resistance to ALS inhibitors in *Echinochloa phyllopogon* (Fischer *et al*, 2000; Osuna *et al*, 2002; Yun *et al*, 2005; Yasuo *et al*, 2009).

Barnyardgrass (*Echinochloa crus-galli*) and late watergrass (*Echinochloa oryzicola*) are exclusively widespread in paddy fields in Korea and have been controlled successfully using ALS and ACCase inhibitors mixed with other herbicides with different modes of action. However, recently ACCase inhibitor resistant *E. crus-galli* was identified (Im *et al.*, 2009) and some of *Echinochloa* species were reported as not well controlled by ALS inhibitors.

Therefore, this study was conducted to confirm if *Echinochloa* species have resistance to ALS inhibitors including azimsulfuron, and to investigate their herbicide resistance mechanism.

MATERIALS AND METHODS

Plant material and growing conditions

Echinochloa crus-galli and *E. oryzicola* accessions suspected to be resistant to ALS inhibitors were collected from Seosan and Gimje in Korea. A reference *E. crus-galli* and *E. oryzicola* as susceptible biotypes were collected in Suwon. Seeds were germinated in the incubator maintained at 33 °C/25 °C (day/night) and left until the first leaf stage. Seedlings at the first leaf stage were transplanted in a plastic pot containing sandy loam soil and grown up to two-tiller stage in the glasshouse at the SNU experimental farm station in Suwon, Korea.

Whole plant assay

Dose-response experiment was conducted in the glasshouse to confirm if *Echinochloa* species are resistant to ALS inhibitors. ALS inhibitors were applied to the suspected R biotypes and the reference S biotypes at a range of doses at the 2-tiller stage by using a compressor-pressurized belt-driven sprayer (R&D Sprayer, USA) equipped with an 8002E flat fan nozzle (Spraying System Co. USA) adjusted to deliver 600 L/ha. Plants were returned to the glasshouse after herbicide application. The experiment consisted of three replicates in a completely randomized design.

Shoot fresh weight was measured at 30 days after application (DAA) and fitted to the standard dose-response model (Streibig, 1980) to estimate GR₅₀ value, the herbicide dose giving 50% growth reduction. R/S ratio was then calculated by dividing GR₅₀ values of suspected R biotypes by those of the reference S biotypes. All statistical analyses were conducted using Genstat 5 (Genstat Committee, 1997).

In vitro ALS enzyme assay

Leaf tissue from *Echinochloa* plants at the 4th-5th leaf stage were harvested at soil level, snap-frozen in liquid nitrogen and stored at -80 °C. The *in vitro* ALS assay was conducted according to the method of Yu *et al.* (2010) with modifications. Enzyme activity was determined colorimetrically (530 nm) by measuring the amount of acetoin formed using

commercial acetoin as a standard. The protein concentration of the crude extract was measured by the Bradford method (Bradford, 1976).

In vitro ALS activity was expressed as percentage of the untreated control. Data were pooled and fitted to the standard dose-response model (Streibig, 1980) to estimate I_{50} values, the herbicide concentration required for inhibiting enzyme activity by 50% of the untreated control. R/S ratio was calculated dividing the I_{50} values of suspected R biotypes by that of the reference S biotypes.

Enhanced herbicide metabolism

It is known that enhanced metabolism-based herbicide resistance is frequently associated with elevated activity of P450s (Siminszky, 2006). Organophosphate insecticides were used as P450s inhibitors to detect enhanced metabolism-based herbicide resistance (Christopher *et al*, 1994; Tardif and Powles, 1999; Fischer *et al*, 2000; Osuna *et al*, 2002; Yun *et al*, 2005; Yasuor *et al*, 2009). Therefore, dose-response experiment was conducted in the glasshouse to evaluate how the P450s inhibitor fenitrothion affects resistance to ALS inhibitors in R biotypes. Herbicides were applied at a range of doses with and without fenitrothion at 500 g a.i./ha applied 5 h prior to herbicide application at the 2-tiller stage by using a compressor-pressurized belt-driven sprayer. All the other procedures were the same as whole plant assay described above.

RESULTS

Whole plant response

To confirm if *Echinochloa* species collected are resistant to ALS inhibitors, ALS inhibitors were foliarly applied to *Echinochloa* biotypes at a range of doses. Gimje biotype showed strong resistance to sulfonylurea herbicide azimsulfuron when compared with the reference susceptible Suwon biotype (Figure 1). The GR_{50} values of Gimje and Suwon biotypes for azimsulfuron were 7863.3 and 21.1 g a.i./ha, respectively. R/S ratio of Gimje biotype was 327.7 (Table 1), suggesting that Gimje biotype is resistant to azimsulfuron. Therefore, Gimje biotype was selected for further investigation of ALS inhibitor resistance mechanism in *Echinochloa* species.

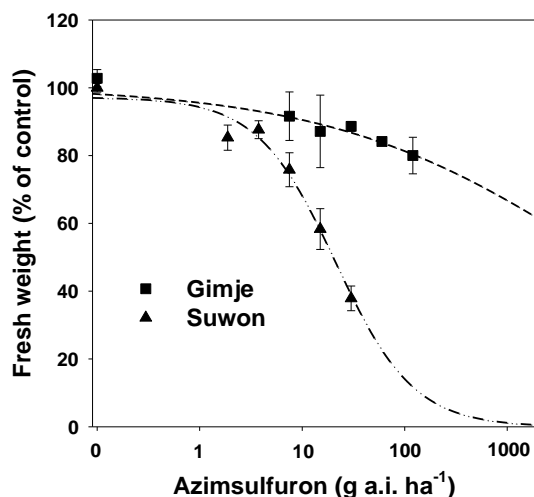


Figure 1. Whole plant dose responses of susceptible (Suwon, ▲) and resistant (Gimje, ■) biotypes of *Echinochloa oryzicola* to azimsulfuron.

***In vitro* ALS activity**

To investigate the mechanism for azimsulfuron-resistant Gimje biotype, *in vitro* ALS enzyme assay using ALS extracted from Gimje and the reference susceptible Suwon biotypes were conducted (Figure 2). The ALS I_{50} values of Gimje and Suwon biotypes for azimsulfuron were 54.4 and 6.3 (μ M), respectively. The I_{50} value of Gimje biotype was 8.7 times higher (R/S ratio) than that of Suwon biotype (Table 1), indicating that ALS inhibitor resistance of Gimje biotype may be due to ALS insensitivity to azimsulfuron. However, resistance of *in vitro* ALS activity was much lower than that of the whole plant response with R/S ratio of 327.7, suggesting that other mechanisms may be involved.

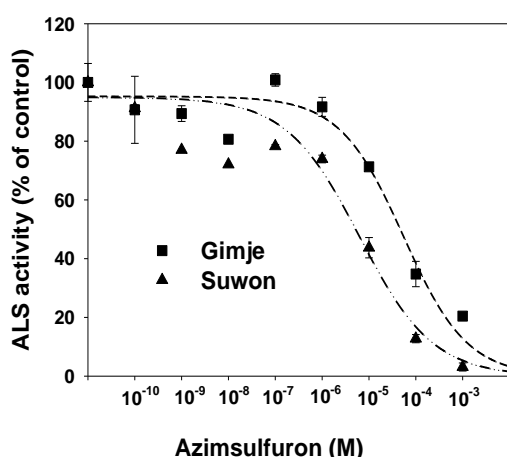


Figure 2. *In vitro* ALS activity of susceptible (Suwon, ▲) and resistant (Gimje, ■) biotypes of *Echinochloa oryzicola* incubated with azimsulfuron.

Enhanced herbicide metabolism

Fenitrothion is one of the organophosphate insecticides that have been used to detect metabolism based-herbicide resistance by the P450s. Fenitrothion (500 g a.i./ha) had no effect on the plant growth of *Echinochloa* species (data not shown). The GR₅₀ values of Gimje biotype for azimsulfuron were 216.2 g and 7863.6 g a.i./ha with and without fenitrothion pretreatment, respectively (Figure 3, Table 1). When compared with that of Suwon biotype, fenitrothion reduced the R/S ratio from 327.7 to 10.2 (Table 1). Therefore, decrease of resistance by fenitrothion would suggest that P450s may be involved in azimsulfuron resistance in Gimje biotype of *E. oryzicola*.

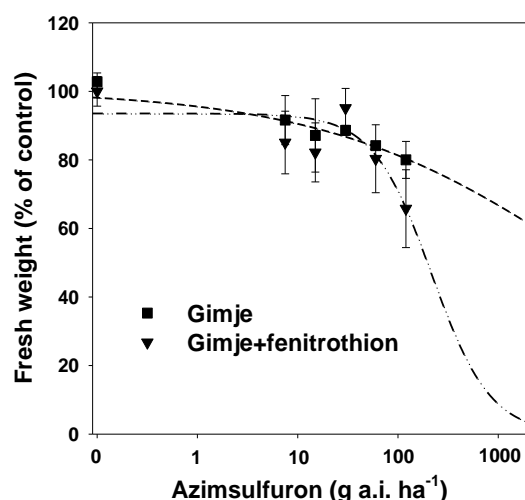


Figure 3. Whole plant dose response of resistant *Echinochloa oryzicola* Gimje biotype treated with azimsulfuron with (▼) and without (■) fenitrothion pretreatment.

Table 1. GR₅₀ values, I₅₀ values and R/S ratios of whole-plant tests and *in vitro* ALS assay with *Echinochloa oryzicola*.

Biotype	Fresh weight (% of control)		ALS activity (% of control)	
	GR ₅₀ value (g a.i./ha)	R/S ratio	I ₅₀ value (μM)	R/S ratio
Suwon (S)	21.1	1.0	6.3	1.0
Gimje (R)	7863.6	327.7	54.4	8.7
Gimje (R)+fenitrothion	216.2	10.2	-	-

In conclusion, our results demonstrate that *E. oryzicola* collected in Gimje has already evolved to be resistant to sulfonylurea ALS inhibitor azimsulfuron, and its resistance mechanism may be due to ALS insensitivity to azimsulfuron and enhanced herbicide metabolism by P450s. Further studies are required to investigate molecular basis of ALS and P450s activities in azimsulfuron-resistant *E. oryzicola*.

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THE EFFECT OF COMPETITION ON MORPHOLOGY AND BIOLOGY OF *AMARANTHUS RETROFLEXUS* L. COHORTS GROWN IN COTTON (*GOSSYPIMUM HIRSUTUM* L.)

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ABSTRACT

Plant morphology determines the reproductive fitness in annual weeds and thus has an important influence on the ability of seeds to germinate and colonize vacant areas. Results from many studies of weed biology suggest that strategic control of weeds is highly dependent on seed production and germination. Empirical studies of seed production have demonstrated that in many species, morphological traits of maternal plants caused by maternal environment can affect seed production and the ability of seeds to germinate in the next generation. An experiment was designed to assess the influence of competition on phenotypic plasticity and the ability of seeds produced to germinate when different cohorts of redroot pigweed (*Amaranthus retroflexus* L.) were grown in cotton (*Gossypium hirsutum* L.). In general, all aspects of plant size in *A. retroflexus* showed similar responses to competition and sowing date. Crop competition significantly ($P < 0.05$) decreased stem diameter, number of leaves per plant, vegetative and reproductive branches in the weed. *Amaranthus retroflexus* plants were tall when grown with the crop and produced leaves with the highest specific leaf area.

Keywords plant architecture, seed germination, redroot pigweed

INTRODUCTION

Plant morphology is a result of the complex interaction between the genetic plan of the species, which determines where meristems are formed, and responses to growing conditions, such as day length and resources, which determine which meristems are activated, aborted and die. Competition from other plants affects resource availability and therefore, indirectly, plant form. The relationship between plant morphology and competition is thus interactive: plant growth form is a response to resource availability, the product of competition between plants, and is a determinant of future resource availability and hence further competition. The outcome of this is usually measured by agronomists and ecologists as biomass or seed production, the summation of the various growth and developmental processes over the whole plant. While hundreds of competition studies have documented how plant density affects crop and weed biomass (for example, Cousens 1985; McLachlan *et al.* 1993; Hashem *et al.* 1998), the effect of density on

morphological features has received less attention (though see Geber 1989). However, a few studies have shown that competition can induce marked phenotypic alterations in the structure of plants, such as plant height, branch number and branch length (Schmid and Harper 1985; Weiner *et al.* 1990). Surprisingly, there are few experimental studies that show the effect of one species, such as a crop, on the morphological traits of a second species, such as a weed. This paper explores variation in plant morphology in the agricultural weed, *A. retroflexus*. The aims are (1) to characterise how plant morphology is influenced by competition and relative emergence time, and (2) the ability of seeds produced to germinate in the next generation.

MATERIALS AND METHODS

The experiment was conducted at the Experimental Station of Darab Faculty of Agriculture and Natural Resources, Shiraz University, Iran in 2009. To provide an array time of weed emergence relative to the crop, *A. retroflexus* was grown at three different sowing dates (0, 7 and 14 days after the crop) among *G. hirsutum* cv. Bakhtegan. Different crop densities (0, 3, 6, 9 plants m⁻²) were used to achieve a range of competition levels. The experimental design was split plot arranged in a randomized complete block with three replicates.

At the end of the growing season, when the growth of the cotton crop stopped and it had no more effect on the weed, *A. retroflexus* plants were cut at their base and taken to the laboratory to measure morphological traits. To determine the effect of crop competition on the germination ability of the weed in the next generation, seed germination of the weed were measured in the laboratory.

All statistical analyses were conducted with SAS software. The significant level was fixed to 0.05 for all analyses. The analysis of variance (ANOVA), with *G. hirsutum* density and sowing date of *A. retroflexus* as the main and sub factors respectively and morphological and biological traits as the response factors, were used to determine the effects of competition and sowing date on morphology and seed germination of *A. retroflexus*.

RESULTS

Amaranthus retroflexus plants grown with the crop were taller whereas those grown in the absence of the crop were shorter (Figure 1a). As crop density increased from 0 to 9 plants/m², stem diameter, number of leaf per plant, in *A. retroflexus* decreased significantly, ($P < 0.05$), (Figure 1a and b). With increasing density from 0 to 9 plants m⁻², the specific leaf area increased significantly, by >25 %. In contrast, number of leaf per plant decreased significantly, ($P < 0.05$), (Figure 1b). *Amaranthus retroflexus* plants produced significantly ($P < 0.05$) more vegetative and reproductive branches at 0 density of the crop than at higher densities (Figure 1c). The mean germination and vigor of the seeds produced by *A. retroflexus* plants grown in the absence of the crop were less than half of that for plants grown in the cotton crop (Figure 1d).

As the sowing date of the weed was progressively delayed relative to the crop, all morphological traits but stem diameter and number of leaf per plant decreased (Table 1). *Amaranthus retroflexus* seeds showed the highest vigor and germination when the maternal plant's sowing date coincided with the crop (Table 1).

DISCUSSION

There were clear differences in the morphological traits of *A. retroflexus* plants grown in mixture compared to those in a widely monoculture. Crop competition and delays in sowing date of the weed significantly ($P < 0.05$) reduced some morphological traits of *A. retroflexus*, such as the number of vegetative and reproductive branches, stem diameter and leaf number per plant. As the sowing date of *A. retroflexus* was further delayed, the number of reproductive branches decreased significantly ($P < 0.05$). Since the morphological traits of plants are known to be a means of seed production in plant species, any changes in plant form potentially affect seed production and traits, such as

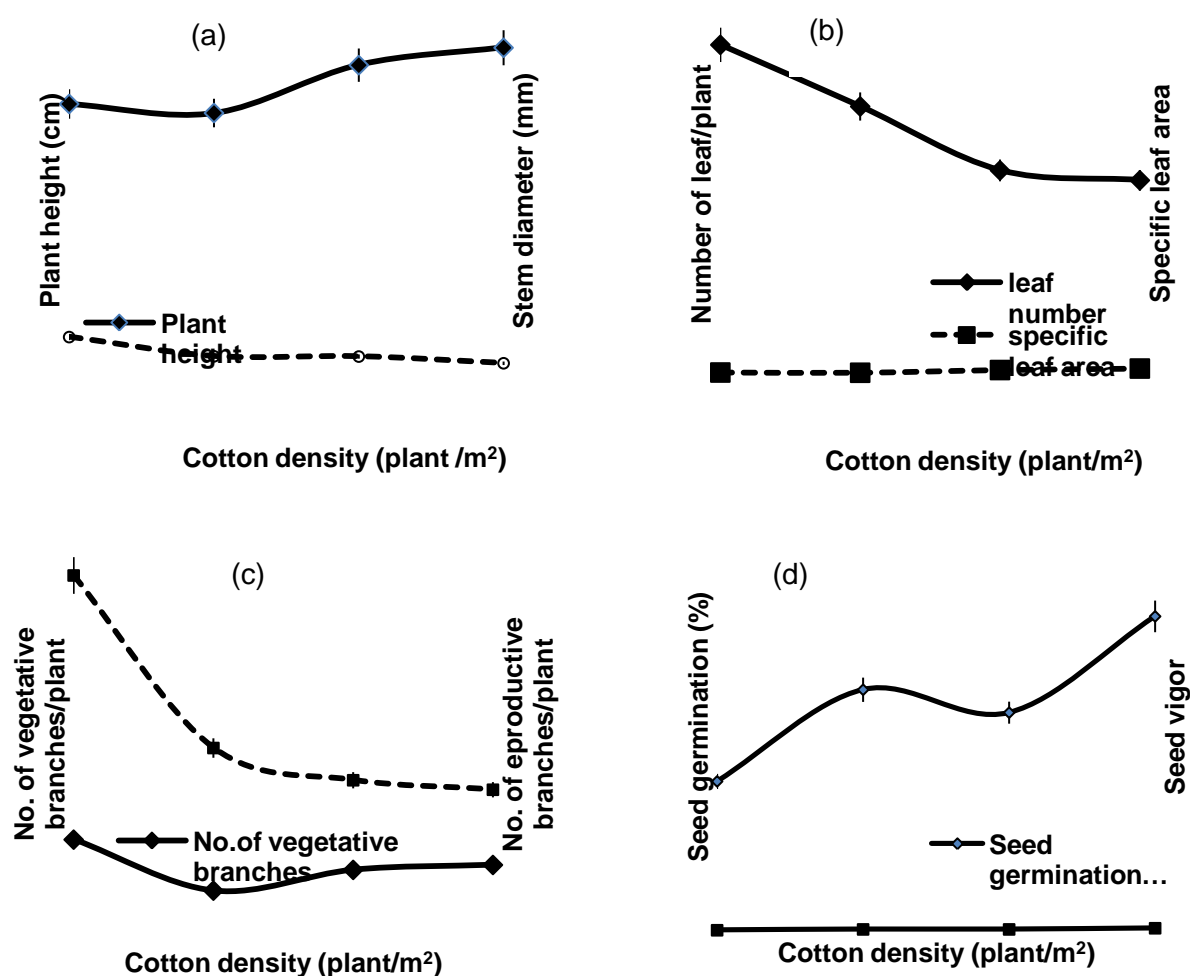


Figure 1. The effect of cotton density on (a) plant height and stem diameter, (b) the number of leaf/plant and specific leaf area, (c) the number of vegetative and reproductive branch per plant, and (d) germination and vigor of the seeds produced in *A. retroflexus*.

Table 1. The effect of sowing date of *A. retroflexus* relative to the crop on morphological traits of the weed and germination of the seed produced. SE is the pooled standard error.

Sowing date (days after crop)	Plant height (cm)	Stem diameter (mm)	Number of leaf/plant	Specific leaf area	Number of vegetative branches	Number of reproductive branches	Seed germination (%)	Seed vigor
0	77.69	11.29	90.69	7.95	13.81	44.07	62.33	0.86
7	75.05	11.31	108.91	7.04	11.68	41.59	54.00	0.83
14	71.28	11.52	124.68	6.25	16.08	39.01	52.83	0.76
SE	2.21	0.57	7.37	0.23	0.87	3.45	0.0094	0.038

seed vigor and germination. The results showed that in the absence of a crop, where there was no competition, *A. retroflexus* had more vegetative and reproductive branches. Having these traits enable the plant to produce more seeds which could be added to the seed bank. The variation in plant morphology with different densities can be explained by phenotypic plasticity and the ability of a single genotype to produce a variety of phenotypes under different environmental conditions. In competitive environments, resources become more limited over time and therefore it is not surprising to find plastic responses in plants to changes in the level of competition, particularly growth and development and thus the final plant form. When grown under competition, resources are allocated to growing taller and as a consequence, axillary growth is reduced, resulting in a thin structure. Similar plastic responses have been reported for other weeds such as wild radish plants grown under monoculture and in mixtures (Cousens, Warringa *et al.* 2001; Taghizadeh and Cousens 2006). These relationships reflect typical plastic responses of plants to changes in light and density/competition relationship (Donohue *et al.* 2000; Smith 2000). It is commonly argued that plasticity in stem elongation is likely to be selected for because taller stems increase light capture in shady environments, while shorter stems promote mechanical stability in open, less competitive environments (Callahan and Pigliucci 2002). These changes in morphology and the germination response of seeds have implications for managing agricultural weeds through managing plant form and seed production.

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DAMAGE CAUSED BY *MERREMIA EBERHARDTII* AND *MERREMIA BOISIANA* TO BIODIVERSITY OF DA NANG CITY, VIETNAM

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ABSTRACT

About 2,000 ha of a special use forest in the Son Tra Nature Reserve and the protected forest at Nam Hai Van Pass, Da Nang City are severely affected by *Merremia eberhardtii*. This invasive woody vine has been rapidly spreading across these protected areas since 1999. In the near-by Ba Na Nui Chua Nature Reserve, about 300 ha of forest are affected by *M. boisiana*. In all locations these woody vines are climbing and covering the forest canopy, killing the trees and plants below by preventing them from absorbing sunlight. The presence of these invasive plants also heightens the risk of forest fires as their large, thick leaves catch fire easily, even when green. Managing these harmful vines is a significant challenge and an important one if native biodiversity is to be conserved. Manual clearing has been applied resulting in the suppression of vine growth, but is not feasible for application over large areas. A management strategy involving chemical and manual removal followed by land rehabilitation is being developed for the long term maintenance of these protected areas.

Key words: *Merremia eberhardtii*, *Merremia boisiana*, invasive woody vine, biodiversity, Da Nang City

INTRODUCTION

Located in central region of Vietnam, a transition region between climate sub zones, biodiversity of the Da Nang city area is assessed as very high, and is best represented in Ba Na Nui Chua Nature Reserve, Nam Hai Van Special Use Forest and Son Tra Nature Reserve. Recently, biodiversity of Da Nang has been severely affected by a range of factors; one of which is environmental weeds.

Since 1999, some thousands of hectares of special use forest in Da Nang City were reported to be severely overgrown by unidentified invasive liana species. The plants have been rapidly spreading across plantation and natural forests. Local researchers from Da Nang Forest Protection Department (FPD) thought that the invading plants were two types of bindweed - *Merremia boisiana* and *Merremia eberhardtii*. The later is considered as the most harmful weed for Da Nang's biodiversity.

Many studies have been conducted for *M. boisiana*, while biological attributes of *M. eberhardtii* still remain unknown. The reason for this is that *M. boisiana* has been recorded

as a dangerous invasive species in southern China. Several studies have revealed the causes of its spread. Eco-physiological research shows that the species is characterized by rapid growth, higher photosynthetic and competition capacity, stronger ability to withstand environmental stresses, and therefore it shows a wide ecological adaptability and is capable of out-competing native taxa. For example, concentrated aqueous extracts of *M. boissiana* could restrain seeds germination of *Brassica parachunensis*, and the bigger the concentration, the stronger the restraint (Zeng et al. 2005). Study on its photosynthetic capacity using gas exchange technique found that the light saturated point and maximal net photosynthetic rate of *M. boissiana* were higher than those of invasive weeds such as *Pueraria lobata* and *Mikania micrantha* (Li et al. 2006). In addition, increasing temperature would cause larger biomass and greater production of *M. boissiana*, resulting in its fast growing and spreading. Thus given climate change predictions one might expect that problems caused by this species will increase in the near future (Zhao et al. 2006).

Since 2000, Da Nang authority has mobilized local efforts and money to chop down, dig up or burn the plant to stop the two species of bindweed from spreading to other areas. However, this method of intense manual control tried on the invader has so far been unsuccessful. This requires a massive amount of manpower, and the overall effect of this remains unclear. Seeking for sustainable methods of management, in 2010 Da Nang authority officially requested the University of Queensland (Australia) to provide technical assistance to deal with these bindweeds. In April 2011, a small team of scientists from the University of Queensland (UQ), led by Professor Steve Adkins conducted a rapid field survey of the *Merremia* species that are invading the natural landscapes around Da Nang City, Vietnam. The aims of the survey were to: (i) identify and confirm taxonomy of the species of *Merremia* invading the landscapes around Da Nang; (ii) assess the extent of the *Merremia* problem in these landscapes; (iii) undertake biological and ecological observations on these *Merremia* species and (iv) to consult with local experts to obtain further information on these species.

MATERIALS AND METHODS

Study areas

The survey was conducted in three protected areas as summarized in the table 1:

Table 1. Locations of the three surveyed sites

No.	Name of protected areas	Coordinates	Districts
1	Son Tra nature reserve	16° 06' - 16° 09'N; 108° 13' – 108° 21'E	Son Tra
2	Nam Hai Van special use forest	16° 10'N 108° 05'E	Hoa Vang and Lien Chieu
3	Ba Na Nui Chua nature reserve	15° 57' – 16° 08'N 107° 49' – 108° 04'E	Hoa Vang

Son Tra Nature Reserve

Son Tra Nature Reserve covers an area of 4,439 ha, including 4,180 ha of natural forest and plantation forest. The Nature Reserve covers a rocky peninsula, situated to the north-east of Da Nang city. Typical vegetation of the Nature Reserve is primary forest, dominated by timber plants of the *Fagaceae*, *Dipterocarpaceae*, *Anacardiaceae*, *Moraceae* and *Sapindaceae* families. Other habitats present at Son Tra include scrub, grassland and plantation forest. Flora of the Nature Reserve consists of 985 plant species, of which 22 species was listed in the Viet Nam red book. Regarding fauna, 287 animal species were listed in the fauna of the Nature Reserve including 36 mammal species, 106 species of bird, 23 reptiles species and 113 species of insect. Among these species, 15 rare species were found (Dinh T.P.A, 1997).

Ba Na Nui Chua Nature Reserve

The Nature Reserve has a total area of 8,838 ha. It is centred on Mount Ba Na, a 1,487 metre-high mountain on the border between Da Nang city and Quang Nam province. The main natural vegetation types present at Ba Na-Nui Chua Nature Reserve are lowland evergreen forest and lower montane evergreen forest. The first forest type is characterised by the presence of tree species in the *Dipterocarpaceae*, while the later forest type is dominated by species in the *Lauraceae*, *Fagaceae* and *Podocarpaceae* families. Areas which have been cleared of forest support scrub or Eucalyptus plantations. The summit of Mount Ba Na was cleared of forest by the use of herbicides during the Second Indochina War and now supports grassland (Hill et al. 1996). Flora of Ba Na NR comprises 793 species, belonging to 487 genera and 134 families. 19 species were listed in the Red book of Vietnam (Nguyen N.T, 2003). Regarding fauna, Ba Na Nui Chua has 256 species, of which 61 species of 26 families belongs to mammal order. 179 bird species (Da Nang statistic yearbook 2009) and 17 reptile species (8 families).

Nam Hai Van Special Use forest

Nam Hai Van is included on the 2010 list as a 10,850 ha cultural and historical site, including 3,550 ha of forest (FPD 1998). The original vegetation of Nam Hai Van would have been lowland evergreen forest below 1,000 m and lower montane evergreen forest above this elevation. However, the natural forest has been extensively cleared by wartime spraying of defoliants, timber extraction, clearance for agriculture and fire. Nam Hai Van lies at the southern extent of the Annamese Lowlands Endemic Bird Area. However, although the avifauna of neighboring Bach Ma National Park has been well studied; less information is available about Nam Hai Van.

METHODS

An initial search and review of the scientific literature regarding *Merremia boissiana* and *Merremia eberhardtii* were done in order to collect information related to biological profiles of these two species.

With the purpose of consultation, collection and collation of relevant information, a seminar was conducted in Da Nang City. Participants included local experts, leaders of relevant departments such as Environment and Natural Resources, Agriculture and Rural Development, and Forest Protection Department. General background of environmental weeds and review of *Merremia* species were presented at the seminar. Information

essential to identify and describe plant communities of the study areas such as physiognomy, floristic composition, vegetation profile, environmental and physical parameters were collected.

A two-day field study was carried out to take appropriate observations, photos and samples. Samples of the two species were collected to consult with eminent botanists at the Hochiminh University of Science to confirm the taxonomy the two species. A flora sheet including species occurrence was used to record the broad floristics.

RESULTS AND DISCUSSIONS

The invading species and invasion situation in Da Nang

The survey confirmed that the *Merremia* species invading Da Nang city are *M. eberhardtii* and *M. boissiana*. Comparison of collected samples and specimens at the National Herbarium (VRM- under management of Institute of Tropical Biology) found that the most serious invasive *Merremia* at the Son Tra nature reserve and the Nam Hai Van special use forest is *M. eberhardtii* (Figure 1), having a white flower. This name was also suggested by Forest Protection Department of Da Nang and Nguyen P.N and Le C. K. from Hochiminh University of Science (Personal communication). The species also is recorded in Thua Thien Hue province (Bach Ma National Park, Truoi Lake and Lang Co).

At Ba Na Nui Chua nature reserve, the invasive *Merremia* species had a yellow flower, and was identified as *M. boissiana* (Figure 2). This species has been reported to be a common invasive species in southern China. Serious damage has occurred to the middle and lower altitudinal regions of the secondary and artificial secondary forests of Hainan Island. Additional, but less intense damage has been reported from Yunnan and Guangxi Provinces in China (Wu *et al.* 2007). The cause of this recent problem in China has been attributed to climate change in this region of China primarily do to an increasing ambient temperature. According to Chinese scientists, *M. boissiana* has three possible centres of origin: firstly in Hainan Island, China, or secondly in Kalimantan Island (Malaysian Sarawak and Eastern Indonesian Kalimantan), or thirdly in the Sa Pa (Lao Cai Province), Tien-Yen (Quang Ninh Province) regions of Vietnam that border the Mekong region of Laos and Guangxi Province, China. Guangzhou, China is now rapidly becoming a new centre of distribution (Wang *et al.* 2005).



Figure 1. *M. eberhardtii* (Photo: Forest Protection Department of Da Nang)

Figure 2. *M. boissiana* (Photo: Forest Protection Department of Da Nang)

About 920 ha of the special use forest in the Son Tra Nature Reserve and 1,100 ha of the protected forest at Nam Hai Van Pass, Da Nang City are severely affected by *M. eberhardtii* (Figure 3 and 4). This invasive woody vine has been rapidly spreading across these protected areas since 1999 (FPD of Da Nang). In nearby Ba Na Nui Chua Nature Reserve, nearly 300 ha of forest are affected by *M. boissiana* (Figure 5). In all locations these woody vines act primarily by climbing up and covering the forest canopy, killing the trees and plants below by preventing them from obtaining sunlight. The presence of these invasive plant species also heightens the risk of forest fires as their large, thick leaves catch fire easily, even when green.



Figure 3. *M. eberhardtii* covering plants in Son Tra Nature Reserve



Figure 4. *M. eberhardtii* climbing the canopy of 20 year old pine trees in the special use forest of Nam Hai Van



Figure 5. *M. boissiana* covering plants in Ba Na –Nui Chua Nature Reserve



Figure 6. Vegetation regeneration after clearing *Merremia* plants

Ecological observations

In the areas where *Merremia* plants have been cleared, many environmental weeds are now present and include: *Vitis pentagona*, *Lantana camara*, *Eupatorium odoratum*, *Bauhinia bracteata* and *Calotropis gigantea* (Figure 6). Therefore, attention needs to be paid to the nature of the vegetation regeneration that occurs after the clearing of the *Merremia* species at the Son Tra Nature Reserve.

Clearing the *Merremia* vines creates a favourable environment for the regeneration of the *Merremia* species from seed in the seed bank as well as many other environmental weeds that have dispersed their seeds to this area. For example, a wild grape species (*Vitis pentagona*) has become well established in this area and is now becoming a new dominant weed species. In addition, it was also noted that seedling regeneration of *M. boissiana* (but not *M. eberhardtii*) was occurring in this area. Seed banks of *Merremia* species, therefore, should be studied carefully to understand the threat of re-introduction in the cleared areas.

Other co-occurring species recorded in the forest areas were: *Grewia asiatica*, *Claoxylon indicum*, *Mallotus hookerianus*, *Ficus hispida*, *Sterculia lanceolata*, *Macaranga denticulata*, *Trema cannabina*, *Millettia dielsiana*, *Pueraria lobata*, *Dalbergia hancei*, *Abrus precatorius*, *Albizia corniculata*, *Lygodium japonicum*, and *Embelia laeta*. There is a need to study the reproductive biology of these co-existing species to understand the effect vegetation clearing has upon these species.



Figure 7. Regeneration of *Merremia* species from seed (believed to be *M. boissiana*).



Figure 8. Rhizome system of *M. eberhardtii*

A native timber species, *Hopea odorata*, is being replanted into the area after the vines are cleared. However, the growth of this species is very slow due to poor soil nutrient content and being smothered by weeds. Therefore, the Management Board of the nature reserves should consider growing other, more faster-growing native shrubs or small trees that can create a ground cover more rapidly, preventing the re-invasion of *Merremia* species, then consider planting the more valuable native tree species at a later date to form a natural forest canopy.

According to Mr. Ho Ngoc Luong, vice head of Lien Chieu district Forest Protection Department, local people have started to use leaves of *M. eberhardtii* to feed their pigs. It

is also considered to be a suitable food source for raising wild pigs. This information may be useful in initiating a study looking into the commercial use of this plant species.

Management measures

Managing these invasive vines is a significant challenge and an important one if the native biodiversity of the region is to be conserved. Manual clearing has been applied resulting in the suppression of vine growth, but this is not feasible for application over large areas. A management strategy involving chemical and manual removal, followed by land rehabilitation needs to be developed for the long term maintenance of these protected areas.

- The efforts of the People's Committee of Da Nang and other Departments concerned in trying to control these invasive plants by manual methods, is to be commended. This method is the most relevant one when no specific information is available on the ecology of the invasive species and information on how other related weedy vines are being successfully managed in other countries.
- Manual clearing needs to be applied until studies can be completed which propose efficient alternative methods for the management of these woody vines. Within the current management practice, the body of the vines needs to be collected and isolated from the ground, to desiccate and then to be burnt. This is a necessary step as decaying vines on the ground surface are likely to release allelopathic substances that may inhibit the germination of native plants.
- In the areas where the extensive growth of *M. eberhardtii* has completely inhibited the growth and development of all other species, chemical control might be used to kill this white-flower species. This method is unlikely to affect the other plant species in the community, but special attention may need to be paid to the effects of the herbicides on the soil characteristics. For the yellow-flowered *M. boissiana* species, the using of herbicides will need to be very carefully applied so not to affect the co-occurring native species in the community.
- *M. eberhardtii* will be extremely difficult to control, as the extensive underground root and rhizome system (Figure 8) will be difficult to remove manually or to be killed with herbicides. However, it will be achieved with persistent management, over a number of years, and if the vine has a limited seed bank with low or no seed dormancy to cause re-infestation.
- Population densities of the vine could be reduced by up to 95% after 3 years of persistent control, with root and rhizome density reduced to less than 1% of the pre-control density after 5 years of control. Furthermore, the recovery of host trees following such control could be fairly rapid if dead vine material is rapidly removed and destroyed.
- Effective control will only be achieved with prolonged effort, both involving manual as well as chemical approaches, coordinated into a sound plan of management.

RECOMMENDATIONS

To expedite the process of management of these woody vine species, there is a need to undertake research. This will need to be undertaken in two phases before any appropriate long term management strategies can be developed that will have resilience under a changing climate.

The main objectives of this short-term research would be: (i) Using two kinds of herbicide trials on small areas of infestation (contact herbicides that target leaves and translocated herbicides attacking the leaves and the underground stem system). Consulting with experts in weed management in Australia and others countries in Asia-Pacific region will generate a list of recommended chemicals that can be safely used to eradicate the plant as well as preferred methods of application. Priority will be given to approaches that kill both stems and roots; (ii) Establishing sample plots where the vines and also secondary weeds are removed in order to monitor the regeneration and the successional change in the plant community. There will be a need to conduct soil seed bank studies to find out what weed seeds are present and what native plant seeds are present and ready to restore the community to the way it was before invasion and; (iii) Conducting a survey of the native flora to look for suitable species for planting in the area when the weeds are removed.

Longer term research would focus on developing a sustainable, long-term management strategy to deal with these woody vines. To do this the following activities would need to be undertaken: (i) Conduct a comprehensive investigation across all of Da Nang and Thua Thien Hue Province to clearly document the distribution of these two invasive species so that the authorities can launch a timely campaign to manage it out over the wider area; (ii) Look at the possibility of using biological control agents to manage these woody vine species. This study will aim to introduce natural enemies from the regions where the vine is native after conducting suitability trial experiments; (iii) Studying physiological and ecological attributes of these species in order to find out the important factors that enable them to become successful invasive species in the area, and to identify weaknesses in their life cycle that may be exploited to obtain better management. The final goal would be to develop an integrated management program for these species and would be robust and effective under a changing climate.

ACKNOWLEDGMENTS

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EVALUATION OF INDIGENOUS FUNGI IN JAPAN FOR THEIR HERBICIDAL ACTIVITY AGAINST JAPANESE KNOTWEED AND GIANT KNOTWEED

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ABSTRACT

The fungi were isolated from diseased plants of both Japanese knotweed (*Fallopia japonica*) in southern Japan and giant knotweed (*F. sachalinensis*) in the north in the wild. Several isolates belonging to *Fusarium*, *Xylaria*, and *Phoma* showed apparent pathogenicity to the knotweed in a primary screening *in vitro*. Among them some isolates belonging to the *Phoma* genus caused severe necrosis on the knotweed seedlings but no symptoms on other plants including the Polygonaceae. The conidia of *P. macrostoma* showed pathogenicity to both species of knotweeds. In addition, our data suggested *P. macrostoma* isolated in Kyushu could be a useful biocontrol agent against invasive knotweeds including the hybrid (*F. x bohemica*).

Keywords: *Fallopia*, biocontrol, *Phoma*

INTRODUCTION

Japanese knotweed (*Fallopia japonica* (Houttuyn) Ronse Decraene), native to East Asia, is a rhizomatous perennial weed belonging to the Polygonaceae family. It was brought into the West from Japan as an ornamental plant in the mid-nineteenth century. The plant spreads quickly to form dense thickets that exclude native vegetation and greatly alter natural ecosystems so that it is widely considered one of the most noxious weeds. The invasive root system and strong growth of the knotweed can damage foundations, buildings, flood defenses, roads, paving, retaining walls and architectural sites (Child and Wade, 2000). Japanese knotweed has been listed by the World Conservation Union as one of the world's 100 worst invasive species (<http://www.issg.org/database/>). Also, the hybrid (*F. x bohemica* (Chrtek & Chrtkova) J.P. Bailey) of Japanese knotweed and giant knotweed (*F. sachalinensis* (F.Schmidt) Ronse Decaene) has appeared in Europe (Bailey *et al*, 1993).

In this study we showed the results of the isolation of fungi both from diseased Japanese knotweed in Kyushu located in southern Japan and from diseased giant knotweed in Hokkaido located in the north, and investigations of fungal pathogenicity to determine the potential as biocontrol agents against troublesome weed in the world.

MATERIALS AND METHODS

Fungal isolate.

Diseased knotweeds showing any symptom such as necrosis on leaves or stem in nature were collected in Kyushu (Kumamoto, Miyazaki and Kagoshima prefecture), and also in Hokkaido. Diseased tissue pieces (approx. 8x8 mm) were taken from the edges of necrotic areas. These pieces were put into 70% ethanol solution for a few seconds, and then transferred to 1% hypochlorite solution for surface-sterilization for several minutes. The pieces were washed in sterile distilled water and placed on Petri dishes containing 1.5% water agar, and kept at 25 C. When fungal hyphae appeared from the pieces on agar plates, the edges of hyphae were picked up by a thin needle under microscope, and transferred on potato dextrose agar (PDA) containing chloramphenicol at 50 – 100 ppm to remove bacterial contaminations. PDA plates were cultured in an incubator at 25 C in the dark for a week. Several fungal characteristics such as growth speed, mycelial color and pigmentation on the plates were recorded for their primary identification.

***In vitro* screening for pathogenicity.**

The isolated fungi were examined for their ability to cause diseases on the knotweeds in a plant box containing wet filter paper. The mycelia agar disks (5 mm in diameter) were placed on detached fresh leaves of the knotweed and on water pepper (*Polygonum hydropiper* L.) grown in a greenhouse, and kept in a growth chamber (12h/12h, day/night) at 25 C for a week.

Conidia production.

Fungal conidia were obtained on V-8 juice agar cultured under black light blue irradiation for 2 - 3 weeks. Conidia of *Phoma* spp. harvested from the surface of agar plates and suspended in 0.1 % Triton X-100 solution were used for further experiments as an inoculum.

Host specificity test.

Both Japanese and giant knotweeds, and common cultivars of water pepper, alfalfa (*Medicago sativa* L.), pea (*Pisum sativum* L.), and potato (*Solanum tuberosum* L.), were examined for the host specificity of the fungi. The conidial suspension (10^6 - 10^7 conidia / mL) was sprayed on the young seedlings of tested plants, which were then kept in dew for 24 hours at 25 C. They were then transferred into a greenhouse and cultivated under greenhouse conditions. Disease symptoms on plants caused by the fungi on the inoculated seedlings were assessed a month after inoculation.

RESULT AND DISCUSSION

Isolation and screening of the fungi.

A total of more than 300 fungal isolates was obtained from necrotic tissues of Japanese and giant knotweeds collected in nature. There were 67 fungal groups, based on the characteristics of the mycelia appearing on PDA. Three genera including *Fusarium*, *Phoma* and *Xylaria* showed an apparent virulence on detached leaves of the knotweed in the primary screening *in vitro* (Fig.2, left side). *Phoma* spp., identified based on their morphological characteristics, were not pathogenic to water pepper belonging to the Polygonaceae, while the isolates of *Fusarium* and *Xylaria* caused necrosis on the plant. The isolates of *Phoma* were re-isolated from the lesions produced after inoculation and fulfilled Koch's postulates so that they were determined to be the causative plant pathogen of the knotweed.

Identification of the pathogen and their herbicidal activity.

The fungal isolates of *Phoma* can grow not only on PDA but also V-8 juice agar medium, and form dark-colored, globose pycnidia within mycelia under irradiation of black light blue. The pycnidia contain numerous conidia, which ooze out in a pale white colored matrix. The characteristics of the fungi (Fig.1) suggested that these isolates are *P. macrostoma* (Boerema *et al*, 2004). The identification was confirmed by DNA sequences.

The fungal host range of *P. macrostoma* isolated from Japanese knotweed and giant knotweed was investigated using the spray inoculation test (Table 1). The isolate from Japanese knotweed in Kyushu killed both species of knotweed and showed herbicidal activity at the concentration of 10^7 conidia /mL (Fig.2, right side). However the isolate from giant knotweed caused visible lesions on leaves of giant knotweed seedlings but mostly no symptoms on Japanese knotweed. Both isolates caused no symptoms at all on alfalfa, pea, potato, and water pepper. These data indicate that *P. macrostoma* has high host specificity on knotweed, and the isolate from Japanese knotweed in Kyushu seems to be a candidate for the biocontrol of knotweeds.

The psyllid, *Aphalara itadori*, has been reported to be effective for the control of knotweeds in Europe (Shaw *et al*, 2009). Also a *Mycosphaerella* fungus has been found in Japanese knotweed as a plant pathogen (Kurose *et al*, 2009). *P. macrosotoma*, isolated in the southern part of Japan, Kyushu, could be another tool for integrated management of this weed. Follow-up experiments, especially field trials, will be needed in the next stage.

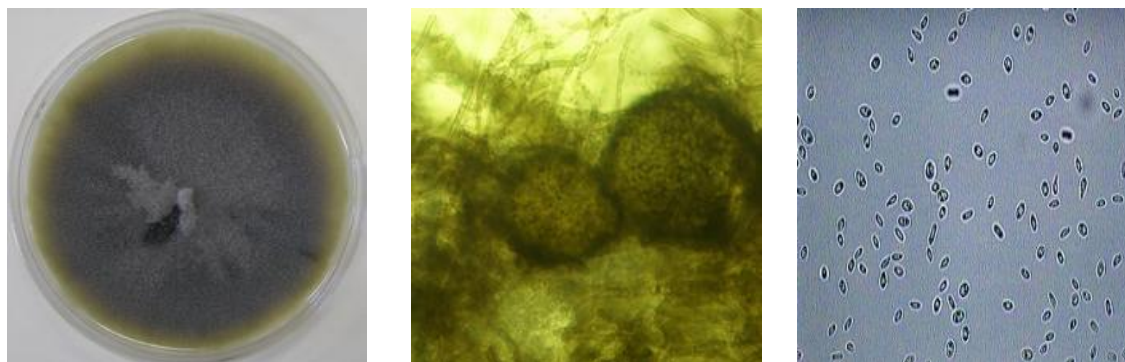


Figure.1 *Phoma macrostoma* isolated from Japanese knotweed. Left, colony on PDA; center, pycnidial formation; right, conidia.



Figure. 2 Pathogenicity of *P. macrostoma* isolated in Kyushu, Japan, to Japanese knotweed. Left, inoculated with mycelia agar disk; right, inoculated with conidia suspension (upper pots).

Table 1. Pathogenicity of *P. macrostoma* isolated from *F. japonica* and *F. sachalinensis*

Host and origin	Japanese knotweed	Giant knotweed	Water pepper	Alfalfa	Pea	Potato
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F. japonica

in Kyushu	+++	+++	-	-	-	-
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F. sachalinensis

in Hokkaido	-	++	-	-	-	-
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+++ , severe to dead; ++, clear symptom; +, slight; -, no symptom

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SELECTIVE EXPOSURE AND EFFICACY OF SULFOSULFURON, SULFENTRAZONE, AND TRIFLOXYSULFURON FOR SEDGE CONTROL IN ESTABLISHED TURFGRASSES

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ABSTRACT

Purple nutsedge (*Cyperus rotundus*), yellow nutsedge (*Cyperus esculentus*) and false-green kyllinga (*Kyllinga gracillima*) are common and troublesome perennial sedge species (Cyperaceae) in turfgrass systems. Although not as widely distributed as other *Cyperus* species, purple nutsedge is a rapidly spreading perennial and has been described as the world's worst weed because it is a serious competitor with more crops in more countries than any other weed. Yellow nutsedge has been described as one of the world's worst weeds and has a greater geographic distribution than purple nutsedge. False-green kyllinga is an aromatic rhizomatous mat-forming perennial, which may tolerate golf course putting green mowing height. Unlike purple nutsedge and false-green kyllinga, yellow nutsedge does not tolerate lower mowing heights. Field experiments were conducted to evaluate sulfentrazone, sulfosulfuron and trifloxysulfuron for postemergent purple nutsedge and false-green kyllinga control. Additionally, greenhouse experiments were conducted to evaluate the effect of selective herbicide placement (soil only, foliage only, or soil + foliage) on sedge shoot number, shoot weight and root weight. Evaluated treatments did not cause objectionable bermudagrass (*Cynodon dactylon*) injury at any time. Sulfosulfuron and trifloxysulfuron provided similar levels of purple nutsedge control (> 60%) while sulfentrazone provided < 30% control. Sulfosulfuron and trifloxysulfuron provided excellent false-green kyllinga control (> 85%), while sulfentrazone provided < 60% control. These results also indicate a sequential application applied 6 WAIT provides the highest level of purple nutsedge and false-green kyllinga control. Further, soil- and soil + foliar-applied herbicides provided the highest level of growth suppression, indicating herbicide-soil contact is required for optimum sedge control. These data confirm sulfosulfuron or trifloxysulfuron offer acceptable postemergent perennial sedge control in tolerant warm-season turfgrasses.

KEYWORDS: purple nutsedge, yellow nutsedge, false-green kyllinga, bermudagrass, sulfosulfuron, sulfentrazone, trifloxysulfuron

INTRODUCTION

Purple nutsedge (*Cyperus rotundus* L.), yellow nutsedge (*Cyperus esculentus* L.) and false-green kyllinga (*Kyllinga gracillima* Miq.) are common perennial sedge species (Cyperaceae) in turfgrass systems. Most nutsedge and kyllinga spp. are C₄ plants (Bryson and Carter 2008; Lin *et al.* 1993) which possess specialized leaf anatomy allowing increased growth and efficiency under high light and temperature regimes (Hattersley 1983; Lin *et al.* 1993; Teeri and Stowe 1976). Purple nutsedge and false-green kyllinga thrive in moist soil conditions (Bendixen and Nandihalli 1987; McElroy *et al.* 2005b) and

tolerate routine mowing. Summerlin *et al.* (2000) concluded that routine mowing at golf course fairway mowing height (1.3 cm) suppresses purple nutsedge growth, although additional control methods are required to provide acceptable levels of control. In contrast, false-green *kyllinga* is able to withstand frequent mowing at heights as low as 1.3 cm (Bryson *et al.* 1997; Summerlin *et al.* 2000). Observations of green *kyllinga* (*Kyllinga brevifolia* Rottb.) and false-green *kyllinga* under golf course putting green mowing height and frequency indicate these species may tolerate any mowing regime in a managed turfgrass system.

The objectives of this research were to evaluate single versus sequential sulfentrazone, sulfosulfuron and trifloxysulfuron applications for purple nutsedge and false-green *kyllinga* control and determine the effect of selective herbicide placement on sedge control.

MATERIALS AND METHODS

Herbicide and Sequential Application. Experiments were initiated to investigate the effect of herbicide, application rate and one versus two applications for purple nutsedge and false-green *kyllinga* control. Purple nutsedge control experiments were conducted on managed turfgrasses at Plymouth Country Club in Plymouth, NC in 2007 and New Bern Country Club in Trent Woods, NC in 2008. Soil type at Plymouth Country Club was a Wahee fine sandy loam (fine, mixed, semiactive thermic Aeric Endoaquults) with 1.3% humic matter (HM) and pH 5.5 (USDA 2010b). Soil type at New Bern Country Club was Tarboro sand (mixed, thermic Typic Udipsamments) with 1.6% HM and pH 5.9 (USDA 2010b). False-green *kyllinga* control experiments were conducted at Hidden Valley Golf Club near Fuquay-Varina, NC in 2007 and The Emerald Golf Club in New Bern, NC in 2008. Three herbicides (sulfentrazone (0.14, 0.28, or 0.42 kg ai/ha), sulfosulfuron (0.033, 0.049, or 0.066 kg/ha) or trifloxysulfuron (0.015, 0.022, or 0.029 kg/ha)) and one versus two applications were evaluated.

Selective Herbicide Placement

Greenhouse experiments were conducted to investigate the effect of soil, foliar and soil + foliar applications of sulfentrazone, sulfosulfuron and trifloxysulfuron on false-green *kyllinga*, purple nutsedge and yellow nutsedge growth. The number of emerged shoots was recorded 30 and 60 DAT. At 30 and 60 DAT, shoots were clipped at the soil surface, dried for 96 h at 60 C and 0% relative humidity and weights were recorded. At 60 DAT, roots were washed free of soil, dried as previously described and weights were recorded. Percent shoot number, shoot weight and root weight reductions, relative to the nontreated, were calculated and reported.

RESULTS AND DISCUSSION

Herbicide and Sequential Application. Reduced rainfall in 2007 likely reduced purple nutsedge and false-green *kyllinga* growth and resulted in overall improved control in 2007 compared to 2008. Cumulative rainfall during trial periods in 2007 was 18.0 cm while 29.1 cm was recorded during 2008. At 12 WAIT in 2007, averaged across herbicide rate and number of applications, sulfosulfuron provided greater purple nutsedge control (92%) than trifloxysulfuron (77%) (Table 1). In 2008, sulfosulfuron and trifloxysulfuron only provided 50 and 58% control, respectively, likely due to increased rainfall in 2008 being more conducive to purple nutsedge growth. Purple nutsedge shoot number reduction followed similar trends as visual estimates of control. Previous research indicates one or two

applications of sulfosulfuron (Baumann *et al.* 2004; Brecke *et al.* 2004; Brecke *et al.* 2007; Harrell *et al.* 2009; Hubbard *et al.* 2007; Marvin *et al.* 2009) or trifloxysulfuron (Brecke *et al.* 2004; Henry and Sladek 2008; Hubbard *et al.* 2007) provide acceptable purple nutsedge control. Purple nutsedge control with sulfosulfuron and trifloxysulfuron in these studies may have been lower due to inclusion of low application rates and single applications. Regardless of year, sulfentrazone provided < 30% postemergent purple nutsedge control or shoot number reduction 12 WAIT, therefore sulfentrazone did not provide adequate control in our experiments (Table 1). Purple nutsedge tubers harvested from nontreated plots were 100% viable (Table 1). Sulfosulfuron reduced purple nutsedge tuber viability the greatest (67 and 49%, respectively, in 2007 and 2008). Trifloxysulfuron also reduced tuber viability compared to the nontreated while tubers harvested from sulfentrazone-treated plots were $\geq 88\%$ viable.

Similar to purple nutsedge, false-green kyllinga control was numerically less in 2008, likely due to increased rainfall (Table 1). In 2007, trifloxysulfuron provided complete false-green kyllinga control and sulfosulfuron provided 91% control 12 WAIT. In 2008, trifloxysulfuron and sulfosulfuron provided 80% and 61% control respectively. Shoot number reduction followed similar trends. These results concur with previous research that reported acceptable false-green kyllinga control with trifloxysulfuron (Breedon and McElroy 2005; McElroy *et al.* 2005a) or sulfosulfuron (Brosnan and Deputy 2008; Unruh and Brecke 2006).

Sulfentrazone did not provide acceptable false-green kyllinga control or shoot number reduction ($\leq 58\%$). Similarly, McElroy *et al.* (2005a) reported 59 and 65% false-green kyllinga control with 0.42 and 0.56 kg/ha sulfentrazone respectively, one year after treatment. These data indicate that adequate false-green kyllinga control may not be obtained each year in turfgrass environments and as previously mentioned may be affected by application rate, inclusion of a surfactant and rainfall, among other factors.

Selective Herbicide Placement

Averaged across species, soil- and soil + foliar-applied sulfentrazone, sulfosulfuron and trifloxysulfuron provided greater shoot number, shoot weight and root weight reduction than foliar applications 60 DAT (Table 3). Further, soil-applied sulfentrazone provided greater shoot number reduction than soil + foliar applications. Soil-applied sulfentrazone reduced shoot number 95% while soil + foliar and foliar applications provided 66 and 5% shoot number reduction, respectively, relative to the nontreated. Foliar-applied sulfentrazone provided minimal (< 15%) shoot and root weight reduction. Soil-applied sulfentrazone likely provided greater reduction because it increased root-available sulfentrazone compared to foliar or soil + foliar applications where sulfentrazone was retained on the foliage. Wehtje *et al.* (1997) reported foliar-applied sulfentrazone had minimal effect on yellow and purple nutsedge and acceptable control required soil contact.

Soil- and soil + foliar-applied sulfosulfuron provided $\geq 85\%$ shoot number and shoot weight reduction and $\geq 77\%$ root weight reduction 60 DAT (Table 3). Foliar applications provided < 45% shoot number, shoot weight and root weight reduction. Sulfosulfuron is an ALS-inhibiting herbicide that is root- and foliar-absorbed (Senseman 2007). Although published research is not available comparing the activity of root- and foliar-applied sulfosulfuron, foliar + soil or soil-only applications of other ALS-inhibiting herbicides have been reported as more effective than foliar-only applications (Williams *et al.* 2001). Further, Richburg *et al.* (1993, 1994) reported soil- and soil + foliar applications of the ALS-inhibiting herbicides

imazethapyr and imazapic reduced yellow and purple nutsedge shoot regrowth greater than foliar applications.

Soil- and soil + foliar-applied trifloxysulfuron provided $\geq 98\%$ shoot number and shoot weight reduction while foliar applications provided less reduction ($< 50\%$) (Table 3). Soil- and soil + foliar-applied trifloxysulfuron provided greater root weight reduction than foliar applications. Although soil- and soil + foliar-applied trifloxysulfuron reduced root weight 86%, harvested roots were partially decayed and were not healthy indicating root weight may have been overestimated. These data indicate soil-applied ALS-inhibiting herbicides are equally effective as soil + foliar applications.

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Table 1. Influence of herbicide on purple nutsedge or false-green kyllinga control, shoot number reduction and tuber or rhizome viability 12 WAIT.^a

Herbicide	CYPRO						KYLGR					
	Control ^b		Shoot number reduction ^c		Tuber viability ^d		Control ^b		Shoot number reduction ^c		Rhizome viability ^d	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
	%											
Sulfentrazone	21	29	19	32	88	89	44	51	58	53	40	21
Sulfosulfuron	92	50	98	65	33	51	91	61	87	79	13	0
Trifloxysulfuron	77	58	88	69	69	71	100	80	98	83	13	0
Nontreated	0	0	-	-	100	100	0	0	-	-	100	100
LSD _{0.05}	5	5	23	16	14	8	6	6	9	15	14	7

^a WAIT, weeks after initial treatment; CYPRO, purple nutsedge; KYLGR, false-green kyllinga; LSD, least significant difference.

^b Percent control based on visual estimates (0 = no visual symptoms; 100 = complete plant death).

^c Percent shoot number reduction relative to nontreated.

^d Percent viability calculated from shoot emergence from harvested CYPRO tubers or KYLGR rhizomes.

Table 2. Influence of sequential herbicide application on purple nutsedge or false-green kyllinga control and shoot number reduction 12 WAIT.^a

No. applications	CYPRO				KYLGR			
	Control ^b		Shoot count reduction ^c		Control ^b		Shoot count reduction ^c	
	2007	2008	2007	2008	2007	2008	2007	2008
	----- % -----							
1	61	33	64	41	68	53	69	57
2	65	59	72	70	89	75	94	86
LSD _{0.05}	NS	4	NS	13	5	6	7	13

^a WAIT, weeks after initial treatment; CYPRO, purple nutsedge; KYLGR, false-green kyllinga; LSD, least significant difference; NS, nonsignificant. Herbicides included sulfosulfuron, sulfentrazone, or trifloxysulfuron.

^b Percent control based on visual estimates (0 = no visual symptoms; 100 = complete plant death).

^c Percent shoot number reduction relative to nontreated.

Table 3. Influence of herbicide and placement on shoot number, shoot weight, and root weight reduction 60 DAT.^a

Placement	Shoot number reduction ^b			Shoot weight reduction ^c			Root weight reduction ^d		
	Sulfent	Sulfo	Trifloxy	Sulfent	Sulfo	Trifloxy	Sulfent	Sulfo	Trifloxy
	----- % -----								
Soil	95	85	99	95	93	100	92	77	86
Foliar	5	12	42	14	23	48	9	44	59
Soil+foliar	66	92	98	83	98	100	83	78	86
LSD _{0.05}	17	22	14	15	14	14	18	11	10

^a Abbreviations: DAT, days after treatment; Sulfent, Sulfentrazone; Sulfo, Sulfosulfuron; Trifloxy, Trifloxysulfuron. Species included false-green kyllinga, purple nutsedge, and yellow nutsedge.

^b Percent shoot number reduction, relative to the nontreated.

^c Percent shoot weight reduction, relative to the nontreated.

^d Percent root weight reduction, relative to the nontreated.

RAPID BIOASSAY METHOD FOR HERBICIDE DOSE-RESPONSE STUDY AND ITS APPLICATION

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ABSTRACT

This study was conducted to develop a rapid bioassay method for herbicide dose-response of *Echinochloa* species and to apply to herbicide resistance diagnosis in *Echinochloa* species. Germinated seeds of *Echinochloa* species were tested in growth pouches containing a range of herbicide concentrations with different modes of action including acetyl-CoA carboxylase, acetolactate synthase, photosystem II, glutamine synthetase and 5-enolpyruvyl shikimate 3-phosphate synthase inhibitors. The growth pouch method was also applied to rapid diagnosis of ALS and ACCase inhibiting herbicide resistance in *Echinochloa* species. Resistant and susceptible biotypes were discriminated at 250-500 mg and 80-120 mg a.i. L⁻¹ of cyhalofop-butyl for *E. crus-galli* and *E. oryzicola*, respectively, and at 700-1500 mg and 1000-2500 mg a.i. L⁻¹ of penoxsulam for *E. crus-galli* and *E. oryzicola*, respectively. Therefore, this method showed a reliable and consistent result with significant time and cost-savings as compared with the conventional dose-response method using whole plants.

Keywords: bioassay, diagnosis, *Echinochloa* species, growth pouch, herbicide resistance

INTRODUCTION

Echinochloa species is one of the most troublesome weeds in rice cultivations both transplanted and direct-seeded. Reliance upon herbicides and continuous use of herbicides with the same mode of action has led to the development of herbicide resistance in weed populations (Holt *et al.* 1993). Studies have been confirmed to be resistant to several herbicides, including acetyl-CoA carboxylase (ACCase) inhibitor, acetolactate synthase (ALS) inhibitor, and photosystem II (PS II) inhibitor in *Echinochloa* species. As far as we know, several dose-response methods of weed species have been developed, including whole plant studies which has been the most widely used for herbicide dose-response and resistance diagnosis. It involves growing plants from seeds collected at the suspect field and spraying them with herbicides applied either by a single or a range of doses. Other methods have also been used. Petri dish assay (Moss 1990; Smeda *et al.* 1996) can be more convenient, cheaper and quicker than whole plant test. Other diagnostic techniques were also used for detecting specific forms of resistance; chlorophyll fluorescence test (Truelove and Hensley 1982; Norsworthy *et al.* 1998), leaf disc flotation test (Hensley 1981; Kemp *et al.* 1990), and pollen germination test (Richter and Powles 1993). However, many of these methods are expensive, limited, difficult to assess, time-consuming, or limited for a certain growth stage. The methods for herbicide dose-response test and resistance diagnosis should be rapid, accurate, cheap,

reproducible and readily available and should provide a steady result on herbicide performance (Moss 1995).

Therefore, this study was conducted to develop a new method by using growth pouch for dose-response test of herbicides with different modes of action. The method was then further applied to herbicide resistance diagnosis in *Echinochloa* specie.

MATERIALS AND METHODS

Whole plant test of herbicides with different modes of action

Pot experiments were conducted to evaluate dose-responses of whole plants of *Echinochloa crus-galli* to herbicides with different modes of action in the glasshouse at Experimental Farm Station of Seoul National University, Suwon, Korea. Herbicides, bentazone (PSII), cyhalofop-butyl (ACCase), bispyribac-sodium (ALS), flucetosulfuron (ALS), penoxulam (ALS), glufosinate (GS) and glyphosate (EPSPS) were applied to *E. crus-galli* at 5th to 6th leaf stage at different dosage ranges using a compressor-pressurized belt-driven sprayer (R & D Sprayer, USA) equipped with an 8002E flat-fan nozzle (Spraying System Co. USA) adjusted to deliver 600 L/ha. All the treatments were replicated three times and were arranged in a completely randomized design. Visual efficacy and above-ground fresh weight were assessed at 30 days after application (DAA), and data were expressed as percentage of the untreated control. GR₅₀ values, dose required to inhibit plant growth by 50% of the untreated control were determined by fitting the data to the standard dose-response model (Streibig, 1980).

Growth pouch test of herbicides with different modes of action

Germinated seeds of *E. crus-galli* were placed in the growth pouch containing a range of concentrations of bentazone, cyhalofop-butyl, bispyribac-sodium, flucetosulfuron, penoxsulam, glufosinate, glyphosate and then kept in an incubation room maintained at 35/25 °C (day/night) with supplementary light where the roots were being kept in the dark. De-ionized water was added to the growth pouch every day to replace water loss due to evaporation. The root and shoot length were measured at 6 days after treatment (DAT). The test consisted of 3 replications of a completely randomized block design.

Diagnosis of ACCase and ALS inhibitor resistant *Echinochloa* spp.

Origin of *Echinochloa* species

Echinochloa crus-galli, Seosan-5, Seosan-46, Seosan-152, and Suwon (reference susceptible) biotypes, previously determined as resistant or susceptible to cyhalofop-butyl (Im et al., 2009) were used for whole plant and growth pouch tests with cyhalofop-butyl. *Echinochloa oryzicola*, Gimje, Iksan, Naju and Suwon (reference susceptible) biotypes, previously determined as resistant or susceptible to penoxulam (Kim, 2010) were also used for whole plant and growth pouch tests with penoxulam.

Whole plant test

Pot experiments were conducted to evaluate dose-responses of *E. crus-galli* and *E. oryzicola* biotypes to cyhalofop-butyl and penoxsulam at a range of their doses. Fresh weight was assessed at 30 DAA and the data were fitted to the standard dose-response

model (Streibig, 1980) to estimate GR₅₀ value of each biotype. The R/S ratio was calculated by dividing GR₅₀ values by the GR₅₀ of the reference susceptible Suwon biotype.

Growth pouch test

Germinated *Echinochloa* seeds were placed in the growth pouch containing a range of concentrations of cyhalofop-butyl and penoxsulam and then kept in the incubation room maintained at 35/25°C with supplementary light with root being kept in the dark. Germination pouches were topped up with de-ionized water every day to replace water loss due to evaporation. The root length was measured at 6 DAT. The test consisted of 3 replications of a completely randomized block design.

RESULTS AND DISCUSSION

Growth pouch method for herbicide dose-response study

Shoot and root growths of *E. crus-galli* were significantly affected by herbicides in both whole plant and growth pouch tests. GR₅₀ values were estimated using shoot fresh weights in the whole plant test and root length in the growth pouch test.

Table 1. GR₅₀ values of herbicides tested by the whole plant and the growth pouch methods.

Mode of action	Herbicide	GR ₅₀ values		
		Whole plant test (g a.i. ha ⁻¹)	Growth pouch test (mg a.i. L ⁻¹)	
		Shoot weight	Root length	Shoot length
PS II	Bentazone	13095.2	2479.0	6066.3
ACCase	Cyhalofop-butyl	15.7	21.4	NA
	Bispyribac-sodium	13.6	193.3	1278.0
ALS	Flucetosulfuron	72.5	2433.3	NA
	Penoxsulam	5.5	6148.7	NA
GS	Glufosinate	113.1	187.2	108.0
EPSPS	Glyphosate	303.4	71.7	345.9

In the whole plant test, the GR₅₀ values for fresh weight of *E. crus-galli* were 13095.2 g, 15.7 g, 13.6 g, 72.5 g, 5.5 g, 113.1 g, and 303.4 g a.i. ha⁻¹ of bentazone, cyhalofop-butyl, bispyribac-sodium, flucetosulfuron, penoxsulam, glufosinate and glyphosate, respectively (Table 1). In the growth pouch test, the GR₅₀ values for root length were 2479.0 mg, 21.4 mg, 193.3 mg, 2433.3 mg, 6148.7 mg, 187.2 mg, and 71.7 mg a.i. L⁻¹ of bentazone, cyhalofop-butyl, bispyribac-sodium, flucetosulfuron, penoxsulam, glufosinate and glyphosate, respectively (Table 1). However, the GR₅₀ values for shoot length was much bigger than those of root length except glufosinate and were not calculable for cyhalofop-butyl, flucetosulfuron and penoxsulam, indicating that roots of *Echinochloa* species are more sensitive to the herbicides tested in this study than shoot except glufosinate. Comparison between the two testing methods thus suggests that the growth pouch test can be used to test herbicide with different modes of action by monitoring root length within a week.

Diagnosis of herbicide resistance in *Echinochloa* spp.

Diagnosis of ACCase inhibitor resistant *Echinochloa* spp.

In the whole plant and growth pouch tests, the fresh weight and root length of resistant and susceptible biotypes were significantly ($P < 0.05$) affected by cyhalofop-butyl. In *E. crus-galli* biotypes, the GR_{50} values by the whole plant test of Seosan-5 and Seosan-152 biotypes were 2.3 and 2.2 times as high as that of Suwon biotype, respectively (Table 2 and Figure 1A). The GR_{50} values by the growth pouch test of Seosan-5 and Seosan-152 were 8.5 and 17.6 times greater than that of Suwon biotype, respectively (Table 2 and Figure 1C).

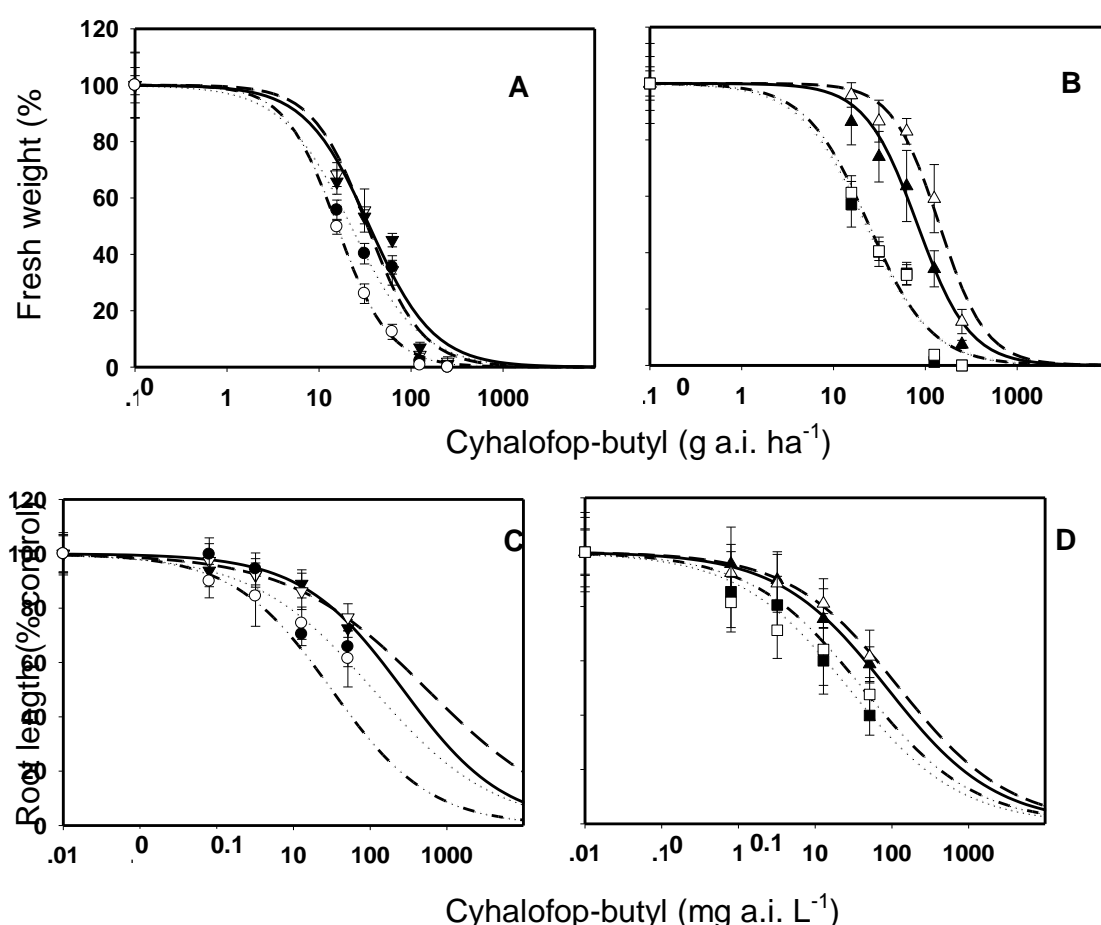


Figure 1. Dose-responses in fresh weight (A, B) of the whole plant test and root length (C, D) of the growth pouch test of resistant (R) and susceptible (S) biotypes of *E. crus-galli* (A, C, Seosan-5 (▼), Seosan-152 (▽), Seosan-46 (●) and Suwon (○)) and *E. oryzicola* (B, D, Gimje (▲), Iksan (△), Naju (■), and Suwon (□)) to cyhalofop-butyl.

In *E. oryzicola* biotypes, the GR_{50} values by the whole plant test of Gimje and Iksan were 3.4 and 5.9 times as high as that of Suwon (Table 2 and Figure 1B). The GR_{50} values by the growth pouch test of Gimje and Iksan were 2.1 and 3.0 times greater than that of Suwon biotype, respectively (Table 2 and Figure 1D). Therefore, these results suggest that the growth pouch test can discriminate between resistant and susceptible biotypes of *Echinochloa* species by measuring root length within a week and can diagnose ACCase inhibitor resistant *Echinochloa* species. The optimum concentrations of cyhalofop-butyl to

discriminate between resistant and susceptible biotypes are 250-500 mg and 80-120 mg a.i. L⁻¹ for *E. crus-galli* and *E. oryzicola*, respectively.

Diagnosis of ALS inhibitor resistant *Echinochloa* spp.

In *E. crus-galli* biotypes, the GR₅₀ values by the whole plant test of Seosan-5 and Seosan-152 biotypes were 4.4 and 2.7 times greater than that of Suwon biotype, respectively (Table 2 and Figure 2A). The GR₅₀ values by the growth pouch test of Seosan-5 and Seosan-152 were 24.5 and 58.0 times greater than that of Suwon biotype, respectively (Table 2 and Figure 2C).

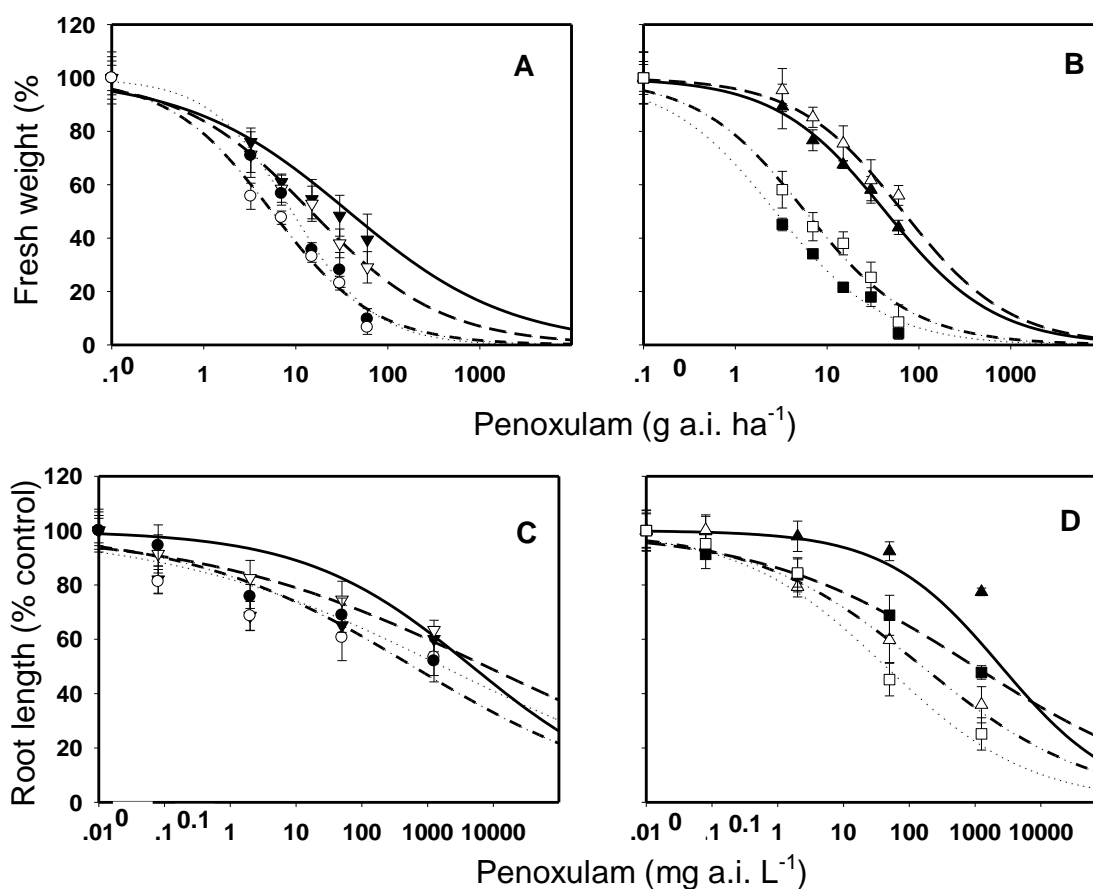


Figure 2. Dose-responses in fresh weight (A, B) of the whole plant test and root length (C, D) of the growth pouch test of resistant (R) and susceptible (S) biotypes of *E. crus-galli* (A, C, Seosan-5 (▼), Seosan-152 (▽), Seosan-46 (●) and Suwon (○)) and *E. oryzicola* (B, D, Gimje (▲), Iksan (△), Naju (■), and Suwon (□)) to penoxulam.

In *E. oryzicola* biotypes, the GR₅₀ values by the whole plant test of Gimje and Iksan were 7.4 and 11.6 times greater than that of Suwon, respectively (Table 2 and Figure 2B). The GR₅₀ values by the growth pouch test of Gimje and Iksan were 5.8 and 6.2 times greater than that of Suwon biotype, respectively (Table 2 and Figure 2D). Therefore, these results also suggest that the growth pouch test can discriminate between resistant and susceptible biotypes of *Echinochloa* species by measuring root length within a week and can diagnose ALS inhibitor resistant *Echinochloa* species. The optimum concentrations of

penoxsulam to discriminate between resistant and susceptible biotypes are 700-1500 mg and 1000-2500 mg a.i. L⁻¹ for *E. crus-galli* and *E. oryzicola*, respectively.

Table 2. R/S ratios in the whole plant and the growth pouch tests of resistant (Seosan-5, Seosan-152, Gimje, Iksan) and susceptible (Seosan-46 and Naju) biotypes of *Echinochloa* species to cyhalofop-butyl and penoxsulam.

Test method	Herbicide	<i>E. crus-galli</i>			<i>E. oryzicola</i>		
		Seosan -5	Seosan -152	Seosan -46	Gimje	Iksan	Naju
Whole plant	Cyhalofop	2.3	2.2	1.4	3.4	5.9	0.9
	Penoxsulam	4.4	2.7	1.7	7.4	11.6	0.5
Growth pouch	Cyhalofop	8.5	17.6	3.5	2.1	3.0	0.7
	Penoxsulam	24.5	58.0	8.6	5.8	6.2	0.3

In summary, the growth pouch method produced accurate and consistent results with significant time and cost-savings as compared with the whole plant test. Moreover, the growth pouch method is cheap and can be conducted with a minimum facility without spray application equipment. In particular, the growth pouch test using germinated seeds requires very little amount of herbicide molecule. Therefore, this method can be used not only for herbicide resistance diagnosis but also for early screening of a new molecule with herbicidal activity in herbicide discovery.

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